Measuring ion transport activities in \textit{Xenopus} oocytes using the ion-trap technique

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Submitted 22 November 2007; accepted in final form 24 September 2008

For the past 20 years, measurement of specific membrane transport activities has been conveniently achieved by expressing individual transport proteins in \textit{Xenopus laevis} oocytes. For electrogenic transport systems, electrophysiology is the method of choice, as it provides sensitive detection of the transport current with excellent time resolution. The two-electrode voltage-clamp technique can be used to study steady-state currents over periods of seconds to hours as well as pre-steady-state charge movements occurring in the millisecond range (4, 7, 18). For electroneutral or weakly expressed transporters, radiolabeled substrate are used for prolonged uptake experiments (minutes to hours). Alternatively, ion-selective microelectrodes (ISE) can be used to measure intracellular ionic activities, but this approach is plagued by poor time resolution due to the large volume of an oocyte and to the intrinsic slow response time of these electrodes (5, 19). Taking advantage of the large size of a \textit{X. laevis} oocyte (1.2 mm in diameter), we developed the ion-trap technique, which uses a large ISE characterized by low impedance (<0.5 GΩ) and a typical response time of 20 ms. As the ISE is gently pushed against the surface of an oocyte, it isolates a small volume of extracellular solution (the trap) in which transport-dependent changes in ionic concentrations can be detected. In this paper, we demonstrate that the ion-trap technique makes it possible to detect steady-state changes in ionic concentration arising from cotransport fluxes as well as fast ion binding/ release events associated with the voltage-dependent reorientation of the ion binding sites of the cotransporter. The data demonstrate that this method can be used to study the transport of ions by both electroneutral and electrogenic transporters with good sensitivity and time resolution.

\textbf{MATERIALS AND METHODS}

\textit{ISE fabrication.} Borosilicate capillaries (1-mm outer diameter × 0.5-mm inner diameter with fibers; EHC, Bowdoin, ME) were pulled using an horizontal puller (Flaming/Brown P-97; Sutter Instruments, Novato, CA), and the tips were broken with forceps, while visualized under a microscope, to provide an approximate tip diameter of 20–100 μm; the tips were then fire polished to create a smooth surface. Larger tip diameters could not be reliably used, as the ion-selective resins were not stably retained within the tip of these ISEs on retracting the ISE from the oocyte surface, which resulted in significant drift in the signal. The microelectrode was briefly dipped in a 1:200 mixture of 90% dichlorodiphenylsilane (Sigma-Aldrich, Oakville, ON, Canada) and 99.9% acetone (Sigma-Aldrich) and then positioned tip-up on a rack and baked at 120°C for an hour. Before use, the electrode was backfilled with a small volume (<1 mm of capillary length) of ion-selective resin using thin capillary tubing and a syringe. Unused silanized electrodes can be stored in a desiccator or in an oven set at 80°C for days without significantly affecting their properties.

\textit{Na+}–selective electrodes were made with a \textit{Na+}–neutral carrier cocktail (cat. no. 71178; Fluka, Oakville, ON, Canada) and were backfilled with 100 mM NaCl. According to the manufacturer, the resin provides an ideal Nernstian response down to 3.2 mM (2), presents a weak Na-to-K permeability ratio ($P_{Na}/P_K = 2.5$), but is insensitive to changes in Ca$^{2+}$ concentration. In our hands, the performance of large-diameter Na$^+$ electrodes was tested by immersing the ISE in a solution containing, in mM, 100 NaCl, 3 KCl, 0.82 MgCl$_2$, 0.74 CaCl$_2$, 10 HEPES and adjusted to pH 7.6 with Tris. Na concentration was reduced to 50, 20, 10, 5, and 2 mM by replacing Na by N-methyl-D-glucamine (NMDG). The signal from a bath KCl flowing electrode (1 M) was subtracted from the ISE signal to correct
for minor changes in liquid junction potentials at the bath agar bridge. For eight different ISEs, the average slope of the electrode signal as a function of log ([Na]) was 51.3 mV/decade from 20 and 100 mM and was slightly reduced to 48.5 mV/decade between 5 and 20 mM (Fig. 1A). Starting from a [Na] of 10 mM, replacing NMDG with choline had no effect, but replacing 80 mM NMDG by 80 mM K produced a positive shift of the ISE signal by 32 mV (n = 6). This is consistent with \( P_{\text{Na}}/P_{\text{K}} \) of 2.03. Sodium-selective electrodes were completely insensitive to the presence of 5 mM \( \alpha \)-methyl-D-glucose (\( \alpha \)MG) or 200 \( \mu \)M phlorizin (Pz), the substrate and specific inhibitor of SGLT1, respectively.

\( \text{H}^+ \)-selective electrodes were made with ISE01 ion-exchanger cocktail (World Precision Instruments, Sarasota, FL) and, according to the manufacturer's data sheet, provide a response of 56 mV/decade from pH 4 to 12 without any significant ionic interferences. \( \text{H}^+ \)-selective electrodes were backfilled with, in mM, 40 K\( \text{H}_2\text{PO}_4 \), 23 NaOH, and 15 NaCl (pH 6.82). In our hands, the performance of large-diameter pH electrodes was tested by immersing the ISE in a solution containing, in mM, 90 NMDG/\( \text{Cl} \), 3 KCl, 0.82 Mg\( \text{Cl}_2 \), 0.74 Ca\( \text{Cl}_2 \), 10 of either MES, HEPES, or Tris. A final pH of 5.5 was reached by mixing the MES-containing solution with the HEPES-containing solution, whereas pH levels of 6, 6.5, 7, and 7.5 were reached by mixing the MES-containing solution with the HEPES-containing solution. In measurements with 11 different ISEs, the average slope of the electrode signal as a function of external pH was 56.8 mV/pH unit (Fig. 1B). As was the case for Na\(^+\)-selective electrodes, \( \text{H}^+ \)-selective electrodes were completely insensitive to the presence of 5 mM \( \alpha \)-MG or 200 \( \mu \)M Pz. For both types of ISE, any electrodes displaying resistances >500 M\( \Omega \) were discarded.

**Oocyte preparation.** Oocytes were surgically removed from *X. laevis* frogs, dissected, and defolliculated as previously described (10). Healthy oocytes were injected with 46 nl of water containing 0.1 \( \mu \)g/\( \mu \)l mRNA coding for human myc-hSGLT1 [as previously demonstrated (3), this epitope-tagged version of human SGLT1 displays properties that are indistinguishable from the untagged form] or 0.1 \( \mu \)g/\( \mu \)l mRNA coding for rat electroneutral \( \text{H}^+ \)/monocarboxylate transporter (MCT1; kindly provided by Dr. Andrew Halestrap, University of Bristol). Oocytes were maintained in Barth's solution [in mM: 90 NaCl, 3 KCl, 0.82 Mg\( \text{SO}_4 \), 0.41 Ca\( \text{Cl}_2 \), 0.33 Ca(NO\( \text{3} \))\( _2 \), 5 HEPES, pH 7.6] supplemented with 5% horse serum, 1 mM pyruvate, 100 U/ml penicillin, 1 mg/ml kanamycin, and 0.1 mg/ml streptomycin for at least 3 days following injection before electrophysiological experiments were performed.

**Solutions.** When needed, \( \alpha \)MG (a nonmetabolized glucose analog) and/or 200 \( \mu \)M Pz were added to the Na\(^+\) buffer (in mM: 90 NaCl, 3 KCl, 0.82 Mg\( \text{Cl}_2 \), 0.74 Ca\( \text{Cl}_2 \), 10 HEPES and adjusted to pH 7.6 with Tris). Sodium replacement was performed with NMDG. Sodium-free acidic solutions (pH 5.5) were buffered with 1.5 mM MES. In Na\(^+\)-free, acidic solutions, the affinity for \( \alpha \)MG is greatly reduced (\( K_{\alpha\text{MG}}^\text{Na} = 20 \text{ mM at } -50 \text{ mV}; \text{ Ref. 20} \)), and 35 mM \( \alpha \)MG was needed to obtain a large cotransport current. Under these conditions, 35 mM \( \alpha \)-mannotol was present in the pH 5.5 solution to prevent osmotic shock when switching to the high \( \alpha \)MG concentration solution; for both solutions, the NaCl (or NMDGCl) concentration was reduced to 65 mM (50 mM mannitol replacement). For studies employing the electroneutral \( \text{H}^+ \)/MCT1, a Na\(^+\) buffer with 1 mM MES (pH 6.5) was used. Unless otherwise mentioned, all chemicals were obtained from Sigma-Aldrich.

**Recording circuitry.** Voltage-clamp recordings and current filtering (1 kHz) were performed using the UC-725C two-electrode voltage-clamp headstage and amplifier (Warner Instruments, Hamden, CT). Data recording was performed using a Digidata 1322A recording system and pCLAMP 8.2 software (Axon Instruments, Union City, CA). The microelectrodes were filled with 1 M KCl and presented resistances <2 M\( \Omega \). Ion-selective measurements were performed with the Duo 773 dual-channel, high-impedance electrometer (World Precision Instruments). The signal from a supplementary 1 M KCl microelectrode placed near the ISE was automatically subtracted from the ion-selective signal to eliminate the voltage drop associated with the bath series resistance. The ion-selective signal (\( V_{\text{ion}} \)) was amplified by the electrometer, digitized, and recorded unfiltered by pCLAMP. A grounded piece of aluminum foil was wrapped around the ISE holder to reduce electrical noise.

**Pulse protocols and data analysis.** A variety of voltage pulse protocols were used with voltages ranging from −155 mV to 40 mV. In all cases, the holding potential was set at −50 mV. When needed, 10–200 identical pulses were averaged by pCLAMP to reduce electrical noise in \( V_{\text{ion}} \). Extra filtering was achieved using the Clampfit low-pass Bessel filter [cutoff frequency (\( f_c \) = 100 Hz). Charge transfer curves were obtained as previously described (10). Briefly, current in the presence of Pz was subtracted from currents measured in the Na\(^+\) buffer lacking Pz. The resultant signal was corrected for steady-state currents by subtracting the mean of a 20-ms time window positioned at the end of the pulse. Integrating this signal yielded the total charge transferred at each potential. Fast \( V_{\text{ion}} \) changes were obtained by averaging 20-ms time windows at 75 or 125 ms after the initiation of the pulse. Slopes were obtained by fitting a straight line to the linear part of the \( V_{\text{ion}} \) vs. time curve. For steady-state measurements of \( V_{\text{ion}} \), a slow linear drift correction was applied to make sure that identical solutions yielded identical \( V_{\text{ion}} \) at the two different times. This drift correction was measured and applied in each individual experiment; its amplitude averaged −0.7 ± 1.2 \( \mu \)V/s (n = 9).
Typical recordings are presented, and similar experiments have been repeated three times or more in oocytes obtained from at least two different donors. All statistical results represent means ± SE.

RESULTS

A *X. laevis* oocyte expressing hSGLT1 was transferred to the bath of a classical two-electrode voltage clamp setup. Current and voltage electrodes were inserted in the oocyte, and the membrane potential ($V_m$) was clamped at $-50$ mV. With the use of a third micromanipulator, the tip of the ISE was immersed in the bath solution and calibrated using extracellular Na$^+$ concentrations of 90 and 10 mM or using Na$^+$-free solutions adjusted to pH 7.5 and 5.5, depending on the selectivity of ISE used. The ISE was then gently pushed against the oocyte vitelline membrane, and a fourth manipulator was used to position a reference KCl electrode within 50 μm of the ISE tip. At this point, two different types of measurement can be performed. To examine steady-state conditions, the trap ionic concentration is measured over time courses of several min as a membrane transport mechanism is stimulated by addition of a cotransported substrate to the bath (this portion of the procedure can be applied to electroneutral transport systems) or by a change in the oocyte holding potential. The ionic concentration measured corresponds to the trap ionic concentration needed to balance the ionic flux through the membrane patch bounded by the ISE with the ionic diffusion across the seal formed at the ISE/membrane junction. To examine initial rate conditions (for electrogenic membrane transport systems only), the transport system is abruptly stimulated by a voltage pulse, and the initial change in the trap ionic concentration is measured. In this case, the ionic gradient between the trap and the bath solution remains almost constant, and the change in the trap ionic concentration reflects exclusively the change in the ionic transport rate in the membrane patch bounded by the ISE.

Steady-state concentration measurements. Figure 2A presents a typical experiment where a Na$^+$-selective electrode was applied against the surface of a voltage-clamped oocyte expressing hSGLT1. The external solution was switched from a normal saline solution, containing 90 mM Na$^+$, to a solution where Na$^+$ had been reduced to 10 mM using NMDG as the replacement cation. As expected, the Na$^+$-selective signal ($V_{Na}$) became more negative and, after 100 s, stabilized to a level that was 53 mV more negative than the value recorded in the presence of 90 mM Na$^+$. Adding 5 mM αMG to the bath solution was associated with a total oocyte current change of $-205$ nA. A drop in the trapped sodium concentration ([Na$^+$]$_T$) could be simultaneously observed from 10.11 to 9.75 mM (Fig. 2A). In a series of eight experiments performed on five different oocytes, we found that, on addition of αMG to the bath solution, an average current of $-245 ± 18$ nA was generated, and the trap $V_{Na}$ decreased by an average of 203 ± 22 μV, corresponding to a $\Delta$[Na$^+$]$_T$ of $-0.080 ± 0.009$ mM.

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**Fig. 2.** Steady-state trapped sodium and pH measurements ([Na$^+$]$_T$, pH$_T$) are shown. A: the solution was first switched from normal saline (90 mM Na$^+$) to a 10 mM Na$^+$ solution. Then, 5 mM α-methyl-d-glucose (αMG) was added to the 10 mM Na$^+$ buffer at $-50$ mV. After reaching a steady [Na$^+$]$_T$ measurement, membrane potential ($V_m$) was stepped from $-50$ to $-90$ mV in 10-mV increments. $V_m$ was then stepped back to $-50$ mV, and αMG was removed. The electrode was calibrated by pulling the electrode from the oocyte surface to measure the true 10 mM value (data not shown). B: correlation between [Na$^+$]$_T$ and αMG current ($I_{αMG}$). The substrate was added at $V_m = -50$ mV, and then the voltage was stepped, in 10-mV increments, to $-90$ mV. All currents in the presence of αMG were corrected for the currents measured in the presence of phlorizin (Pz) and were fitted with a straight line. C: steady-state surface pH (pH$_S$) measurement. The solution was switched from pH 7.6 to pH 5.5. When a steady pH$_S$ was reached, 35 mM αMG was added to the bath. $V_m$ was then stepped to $-90$ mV in 10-mV increments and returned to $-50$ mV. αMG was removed from the bathing solution, and Pz was added. The ion-selective microelectrode (ISE) was then pulled away from the oocyte to measure the pH 5.5 value (arrow). Data was reduced to yield 1 point per second. The 3 vertical interruptions in the current trace represent periods where series of voltage pulses were applied to the oocyte. D: comparison of pH$_S$ and $I_{αMG}$. Currents in the presence of αMG were corrected for the currents measured in the presence of Pz. The substrate and inhibitor were added at $-50$ mV. $I_{m}$, membrane current; $I_{αMG}$, αMG-sensitive current.
Please note that this type of experiment can be performed with an electroneutral transport mechanism. In the experiment shown, the drop in $[\text{Na}^+]_{\text{T}}$ was further increased by changing $V_m$ in 10-mV steps from $-50$ mV to $-90$ mV. After the total oocyte current was corrected for the presence of a current, unrelated to SGLT1 (the current in the presence of Pz was measured at each $V_m$ before the experiment), a linear correlation was observed between the change in $[\text{Na}^+]_{\text{T}}$ and the voltage-dependent change in the cotransport current (Fig. 2B). In the absence of αMG, the Pz-sensitive current (the so-called leak current) measured at $-50$ mV is minimal, and $[\text{Na}^+]_{\text{T}}$ reached the value predicted by the linear relationship between the Pz-sensitive current ($I_{\text{Pz}}$) and $[\text{Na}^+]_{\text{T}}$.

The same type of experiment can be performed using $\text{H}^+$ to activate SGLT1 and a $\text{H}^+$-selective electrode to measure the trap $\text{H}^+$ concentration (reported here as $pH_T$) (12, 20). Figure 2C depicts such an experiment performed in the absence of Na$^+$ and with a pH 5.5 external solution weakly buffered with 1.5 mM MES. In the absence of αMG, SGLT1 exhibits an inward leak current, which is thought to originate from an uncoupled proton influx (20); this partially explains why $pH_T$ stabilized at 5.61, i.e., a slightly more alkaline level than the bath solution. When protons supply the driving force, the affinity for αMG is reduced, and a high sugar concentration must be present to generate a significant cotransport current. When 35 mM αMG was added to the bath, an additional inward current of $-188$ nA was generated, and $pH_T$ alkalinized from 5.61 to 5.73. With αMG present, the voltage was then stepped, in $-10$-mV increments, to $-80$ mV. When 200 μM Pz was added to the bath, the leak current disappeared (leak current $\approx$ $-50$ nA at $-50$ mV), and the steady-state $pH_T$ changed from 5.61 to 5.56 within 20 s. The steady-state $pH_T$ value reached at each voltage is plotted in Fig. 2D and was proportional to the αMG-stimulated cotransport current. The same experiment was performed five times with two different ISE on five different oocytes yielding the following average results: 1) in the presence of pH 5.5 buffer, $pH_T$ averaged 5.66 ± 0.04; 2) addition of 200 μM Pz acidified $pH_T$ to 5.59 ± 0.02 and demonstrated an average Pz-sensitive leak current of $-86$ ± 20 nA; and 3) perfusion with 35 mM αMG generated an average inward current of $-313$ ± 44 nA and was associated with trap alkalization to pH 5.85 ± 0.07.

To illustrate use of the ion-trap technique with electroneutral transporters, a $\text{H}^+$-selective electrode was used to detect proton fluxes associated with the $\text{H}^+$/lactate$^-$/cotransporter MCT1 (11). As shown in Fig. 3, a pH-ISE was brought into the vicinity of a nonvoltage-clamped oocyte expressing MCT1. When the ISE was put in contact with the oocyte, the pH signal rapidly moved from 6.52 to 6.62. This effect can also be observed with SGLT1-expressing oocytes as well as with noninjected oocytes and must be due to an endogenous proton influx or to a reaction of the ISE on establishing physical contact with the oocyte vitelline membrane. After 1–2 min, the pH signal was stabilized, and a solution containing 0.2 mM lactate was introduced into the bath. The trap pH increased to 6.72 within 20 s. This alkalization of the trap reflects a lactate-induced proton influx that is decreased by successively reducing the bath lactate concentration to 0.1 and 0 mM. When the ISE is pulled away from the oocyte, the bath pH was correctly measured, and a calibration was performed (during the recording interruption in Fig. 3). Finally, the constant pH of all solutions used was checked directly with the ISE in the experimental chamber. Addition of 0.1 mM lactate produced alkalinization by 0.093 ± 0.015 pH unit, whereas addition of 0.2 mM lactate produced additional alkalinization by 0.086 ± 0.009 pH unit ($n$ = 5 oocytes). Lactate addition produced no significant effects in measurements using noninjected oocytes (data not shown).

### Fast $\Delta[\text{Na}^+]_{\text{T}}$ Measurements

Figure 4A shows the membrane current ($I_m$) and $V_{\text{Na}}$ during 150-ms duration $V_m$ pulses. A voltage-dependent slope clearly appears in the $V_{\text{Na}}$ tracings when $V_m$ is abruptly changed in the presence of 5 mM αMG. For negative $V_m$ pulses, $V_{\text{Na}}$ becomes progressively more negative with time, which is consistent with Na$^+$ leaving the trap by entering the cell through the patch of membrane bounded by the ISE. When αMG is removed, the slope disappears and is replaced by a steady-state value of $V_{\text{Na}}$, which varies by $\sim 300$ μV from the most negative ($-140$ mV) to the most positive (+25 mV) $V_m$ levels. Adding 200 μM Pz further reduces the steady-state change in $V_{\text{Na}}$ by 50%. Figure 5 shows the average instantaneous slope (ρ) of the $V_{\text{Na}}$ vs. $t$ curve along with the current caused by the addition of 5 mM αMG ($I_{\alphaMG}$) for a series of eight oocytes. The slopes were measured between $t = 30$ ms and $t = 150$ ms at each membrane potential studied. As shown in Fig. 5, the ρ vs. $V_m$ curve is perfectly parallel to the $I_{\alphaMG}$ vs. $V_m$ curve, which indicates that the ion-trap technique is capable of detecting the minute changes occurring in $[\text{Na}^+]_{\text{T}}$, which are due to activation of the cotransport mechanism within the 150-ms duration of the voltage pulse. In the absence of αMG, time-independent changes in $V_{\text{Na}}$ are observed when the potential is stepped to different levels. A major portion of this signal is related to SGLT1 as it disappeared in the presence of Pz (Fig. 4). In the presence of Pz, the remaining change in $V_{\text{Na}}$ was found to be proportional to the total oocyte current ($I_m$) (Fig. 6A). The relation between $I_m$ and $V_{\text{Na}}$ was also observed to be linear when control (not injected with mRNA) oocytes were used (Fig. 4B). This suggests that the remaining sources of change in $V_{\text{Na}}$ are due to a constant resistance multiplied by the current circulating at any moment between the oocyte and the bath current electrode. We hypothesized that this voltage drop could occur through the loose seal resistance created by the contact between the ISE and the oocyte membrane. If an average ISE covers 1/2,000th of the total oocyte geometrical surface, the potentials presented...
in Fig. 6A would thus be due to the measured $I_{in}$ divided by 2,000 and then multiplied by a resistance of 0.5 MΩ. This appears reasonable for a seal resistance between a fire-polished pipette of >50 μm in diameter at the vitelline membrane of an oocyte. Recordings were corrected for this local voltage drop across the seal resistance created by the ISE. To do so, an effective resistance was measured by fitting a straight line through the $V_{ion}$ vs. whole oocyte current in the presence of the inhibitor for each set of recordings. Data were corrected by subtracting this effective resistance multiplied by the whole oocyte current during each condition. In the absence of αMG, these corrected $V_{Na}$ were used to accurately estimate the sudden change in $[\text{Na}^+]_T$ that was associated with the nonco-transport activity of SGLT1 at different potentials. This value, measured in mV ($\Delta V_{Na}$), is called “the Step” and is presented in Fig. 6B as a function of $V_m$ for a series of eight oocytes. This amount of Na$^+$ that rapidly moves in or out of the trap on changing $V_m$ in the absence of αMG is reminiscent of the pre-steady-state charge movements ($Q$ vs. $V_m$ curve) that have been observed for a variety of cotransporters and pumps (8, 13, 15, 18). For the same series of eight oocytes, the $Q$-$V_m$ curve was measured by integrating Pz-sensitive currents ($I_{Pz}$, A), and the average data are presented in Fig. 6B. In the presence of 10 mM Na$^+$, the $Q$-$V_m$ curve does not reach a minimum within the experimental range of negative $V_m$. Nevertheless, it could be fitted with a Boltzmann curve:

$$Q = \frac{Q_{hyp} - Q_{dep}}{1 + e^{V_m/V_{0.5}}} + Q_{dep}$$

Under these conditions, if $Q_{dep}$ is set to 5 nC, the best parameter values are: $V_{0.5} = -164 \pm 21$ mV, $Q_{hyp} = -50 \pm 14$ nC, and $dV_m = 48 \pm 3$ mV. The Boltzmann fit of $Q$ shown in Fig. 6B is superimposed onto the Step vs. $V_m$ curve, and it can be seen that the two parameters vary across membrane potentials almost identically with each other. This is consistent with the hypothesis that a rapid change in $V_m$ generates an electrogenic conformational change that can be observed as the

Fig. 4. Initial rate measurements of $[\text{Na}^+]_T$ during voltage pulses are shown. $A$: the signals presented are averages of 10 series of the voltage pulses shown. Currents and $V_{Na}$ were low-pass filtered at 1 and 0.1 kHz, respectively. Bath Na$^+$ is 10 mM, and pH is 7.6. $V_{Na}$ is shown uncorrected for series resistance artifacts. When 5 mM αMG was added to the bath, a slope appeared in the $V_{Na}$ signal. Pz abolished the Na$^+$ glucose cotransporter (SGLT1)-mediated pre-steady-state currents and reduced the amplitude of $V_{Na}$ below the level seen in the Na$^+$ buffer. $B$: initial rate $[\text{Na}^+]_T$ measurement for a noninjected oocyte.

Fig. 5. Slope (p) and current ($I_{AMG}$) comparison for multiple experiments using Na$^+$ electrodes is shown. The slope, during a voltage pulse, is obtained by fitting a straight line to the linear part of $V_m$, and is 0 at $-50$ mV. The average slopes for 8 experiments are shown with interpolation. The $I_{AMG}$-dependent current was obtained by averaging a 20-ms time window at the end of the leak-corrected signal. The mean αMG-dependent currents for 8 experiments are shown with interpolation.

Fig. 6. Series resistance correction and comparison of charge transfer (Q) and Step ($V_{Na}$) curves are shown. $A$: the apparent resistance was obtained by fitting a straight line through the $V_{Na}$ vs. $I_{Pz}$ data. These values were obtained at holding potentials ranging from −140 to 40 mV by averaging a 20-ms time window at the end of the pulse (dashed boxes). $B$: the charge transfer ($Q$) was obtained by integrating the Pz-sensitive pre-steady-state currents. $Q$ is shown with its Boltzmann fit superimposed (solid line). After the correction of $V_{Na}$ with the apparent resistance obtained as described in $A$, a 20-ms time window was averaged at the end of the pulse. The average Step derived from 8 experiments is shown on the right with the Boltzmann fit of $Q$ normalized and superimposed (dashed line).
Subsequently, we chose to estimate the $\Delta[\text{Na}^+]_T$ movement that can be specifically attributed to the Step. Convolution of the voltage pulses makes it difficult to precisely estimate the steady-state proton flux. The presence of a slope during the voltage pulse (which includes the Step and the slope contribution) and the value recorded 100 ms after $V_m$ was returned to $-50$ mV where the oocyte leak current and $V_H$ slope are observed to be negligible. In the presence of Na, the same treatment proved effective in giving the same $\Delta[\text{Na}^+]_T$ as when it was calculated from the levels measured during the pulse.

**Control experiments.** Several control experiments were performed to confirm that the measured changes in $V_{ion}$ were a direct reflection of the cotransporter activity. First, the slopes measured during a voltage pulse are $P_z$-sensitive (Figs. 4A and 7A) and are absent in noninjected oocytes (Figs. 4B and 7B). Both the slope and the Step disappear when the electrode is pulled slightly away from the oocyte surface. Moreover, the Na$^+$ Step disappears when the bath Na$^+$ is increased to 0.2 mM $P_z$.

**Fast $\Delta pH_T$ measurement.** The same analysis of rapid SGLT1-mediated changes in cation concentration can be performed when protons are used to drive the glucose cotransporter in the absence of sodium. Figure 7 shows a typical experiment performed with a $H^+$-selective electrode in Na$^+$-free buffer at pH 5.5. There is clear evidence for a slope in the presence of 35 mM $\alpha$MG between $t = 60$ ms and $t = 150$ ms. In the absence of glucose, there is a significant leak ($I_{ion,\alpha MG}-I_{PZ}$), which, at $-140$ mV, corresponds to $\sim 14\%$ of $I_{ion,\alpha MG}$. In agreement with the presence of a relatively large leak, the potential of the pH ISE ($V_H$) vs. time curve in the absence of $\alpha$MG is characterized by a Step and a certain slope during the time course of the pulse duration. The complete experimental series was performed on three oocytes and includes recordings obtained with 0, 2.5, and 35 mM $\alpha$MG and in the presence of 0.2 mM $P_z$. Once again, there was a strong similarity between the whole oocyte cotransport current ($I_{ion,\alpha MG}$) and the slope of the $V_H$ vs. time curve for each of the two $\alpha$MG concentrations (Fig. 8A). In the absence of $\alpha$MG, the Step, measured between $t = 130$ ms and $t = 150$ ms, is proportional to the transferred charge, obtained by integrating the $P_z$-sensitive current over time (Fig. 8B). Because of the nonnegligible leak current observed at pH 5.5 (Fig. 7), the $V_H$ vs. time curve included both a Step and a slope, which is associated with a significant steady-state proton flux. The presence of a slope during the voltage pulses makes it difficult to precisely estimate the amplitude that can be specifically attributed to the Step. Consequently, we chose to estimate the $H^+$ Step by measuring the difference between the value of $V_H$ recorded at the end of the voltage pulse (which includes the Step and the slope contribution) and the value recorded 100 ms after $V_m$ was returned to $-50$ mV where the oocyte leak current and $V_H$ slope are observed to be negligible. In the presence of Na, the same treatment proved effective in giving the same $\Delta[\text{Na}^+]_T$ as

![Fig. 7. Simultaneous oocyte current $I_m$ and pH$_T$ measurements ($V_{H}$) in a pH 5.5 buffer are shown. A: the signals shown were selected from a series of voltage pulses that were repeated 8 times for averaging purposes. Currents and $V_{H}$ were low-pass filtered at 1 and 0.1 kHz, respectively. $V_{H}$ is shown uncorrected for series resistance artifacts. In the pH 5.5 buffer, a significant pre-steady-state associated $\Delta pH_T$ (Step) appears with a nonnegligible, leak-associated slope. The signal is completely $P_z$-sensitive. B: initial rate pH measurements for a noninjected oocyte exposed to 35 mM $\alpha$MG.](http://ajpcell.physiology.org/)

![Fig. 8. Comparison of electrophysiological steady-state and pre-steady-state current measurements with the observations provided by the ion-trap technique is shown. A: average $\alpha$MG-sensitive current ($I_{ion,\alpha MG}$) with the slope ($\rho$) measured in the $V_{H}$ vs. $t$ recording in the presence of 2.5 and 35 mM $\alpha$MG ($n = 3$). The interpolation of $I_{ion,\alpha MG}$ at each concentration (full line) was normalized and superimposed on the $V_{H}$ slope curve (dashed line). B: average charge transfer ($Q$) obtained from the integration of the pre-steady-state transient currents ($n = 13$) with its Boltzmann fit superimposed (solid line) and average corrected Step in the $V_{H}$ vs. $t$ recording ($n = 18$) with the normalized Boltzmann fit of $Q$ superimposed (dashed line).](http://ajpcell.physiology.org/)
mM. This is expected since the electrode response is logarithmic, and the ratio between the cotransporter signal amplitude and the ambient Na\(^+\) concentration becomes negligible at high Na\(^+\). In contrast, the ohmic contribution from the patch current multiplied by the seal resistance (Fig. 6A) remains intact when Na\(^+\) is increased. With a H\(^+\)-selective electrode, the slope of \(V_H\) in the presence of ionic concentration in the trap when the flux across the membrane is exactly balanced by diffusion across the seal resistance, i.e., measuring the change in ionic concentration of 0.4%.

Confirmation experiments were also performed with the human H\(^+\)/myo-inositol transporter (HMIT) where we observed a clear slope in \(V_H\) vs. time measurements when in the presence of myo-inositol and an acidic buffer.

**DISCUSSION**

*Detection limits.*Traditionally, ISEs have tip diameters much smaller than 1 \(\mu\)m and present impedances on the order of tens of \(\Omega\) (9). They are characterized by a high electrical noise level and slow rise time. To overcome these limitations, we used large ISEs. We quickly found that ISEs with tips >100 \(\mu\)m do not hold resin tightly enough to perform long experiments, whereas tips <20 \(\mu\)m exhibited an unacceptably high resistance (>500 \(\Omega\)), which compromised the sensitivity and the time resolution of the technique. We thus settled on ISEs in the 20–100 \(\mu\)m range. We routinely achieve ISE impedances as small as 100 \(\Omega\), thus reducing their characteristic response time to ~20 ms (Figs. 4 and 7). More than 70 ISE were fabricated during the time course of the present study, and, in our view, their characteristics are reproducible, and the variability between experiments stems primarily from the size of the trap and the resistance of the seal rather than from the performance of the electrode used. If the signal is filtered with a low-pass Bessel filter with a 1-kHz cut-off frequency, a typical noise level of 0.1 mV can regularly be achieved. When used to detect ion concentration changes within a 150-ms voltage pulse, the cut-off frequency can be reduced to 100 Hz, and the noise level can be lowered to ~0.04 mV. The noise level can be further reduced by a factor of 3-to-10 by averaging 10–100 identical voltage pulses. For nonelectrogenic transporters, steady-state measurements must be used, and the time response is limited by the time typically required for the extracellular solution to gain access to the trap (~20 s). Under these circumstances, the ion-trap signal can be digitized at 100 Hz, and local averaging can be performed to yield 1 point per second displaying a noise level of ~10 \(\mu\)V. The sensitivity of the signal is then limited by spontaneous fluctuations reaching 0.1 mV in amplitude over periods of minutes (Fig. 2A). For an ideally selective ISE, a sensitivity of 0.1 mV corresponds to a change in ionic concentration of 0.4%.

*Steady-state measurements.* The ion-trap technique was first used to examine steady-state conditions, i.e., measuring the ionic concentration in the trap when the flux across the membrane is exactly balanced by diffusion across the seal resistance between the ISE and the plasma membrane. In the absence of Na\(^+\), [Na\(^+\)]\(_T\) is very close to the bath Na\(^+\). Addition of glucose generates a shift in \(V_{Na}\), which can be resolved by this technique as indicated by the small SE associated with this measurement (~0.080 ± 0.009 mV). Note that, under steady-state conditions, the transporter need not be electrogenic, and any transporter mediating a Na\(^+\) flux of 7 mmol/h in an oocyte would generate the same signal. Employing an electrogenic transporter allowed us to change the ionic flux rapidly through application of different membrane potentials. When \(V_m\) is changed from -50 to -90 mV in 10-mV increments, Fig. 2, A and C, as well as Fig. 3 show that <20 s are required for a new equilibrium to be reached between the trap and the bath and that the steady-state value reached after this short delay is proportional to the cotransport current.

Steady-state measurement can also be performed while measuring external pH (pH\(_T\)) in the absence of Na\(^+\). On changing the perfusion solution from a pH 7.5 buffer to a pH 5.5 buffer, pH\(_T\) became more acidic but stabilized before reaching pH 5.5. This steady-state difference is due to the presence of a significant leak current mediated by SGLT1 at acidic pH. This is confirmed by the fact that Pz perfusion inhibited the leak current and induced a further acidification of the trap that progressively tended toward bath pH (Fig. 2, B and D). This indicates that the leak current, in the absence of Na\(^+\), is indeed mediated by proton transport. This is a significant observation as the ionic nature of the leak current through SGLT1 remains poorly understood. Addition of Na\(^+\) generated a large inward current and concomitant alkalinization of the trap. Figure 2D shows that the pHT, reached at steady state, is proportional to the Na\(^+\) cotransport current. With H\(^+\) fluxes in the presence of a weakly buffered extracellular solution (1.5 mM MES), the method detection limit would correspond to a change in cotransport current of 20–50 nA, which corresponds to a proton flux of 0.7–1.8 mmol/h per oocyte.

*Initial rate measurements.* The ion-trap technique can also detect voltage-dependent changes in the trap ionic concentrations over the initial 20–150 ms of a voltage pulse. During this very short period, the ionic concentration in the trap is far from attaining a new steady-state value as ~20 s would be required to achieve this. We are thus dealing with initial rate conditions where the change in the trap ionic concentration reflects a change in the ionic transport across the membrane covered by the ISE with minimal changes in the ionic flux across the seal between the trap and the external solution. The changes in \(V_{ion}\) are rather small over such short time periods, and a correction needs to be applied for the voltage drop across the seal resistance. This is best measured in the presence of Pz such that no SGLT1-dependent ion fluxes can occur across the patch of membrane covered by the ISE. Under these conditions, there remained a voltage-dependent change in \(V_{ion}\) that was proportional to the total oocyte current (Fig. 6A). This \(\Delta V\) is thought not to represent any change in the local cationic concentration since it is still present when the absolute sensitivity of the electrode is decreased by raising the ambient cationic concentration well above detectable limits (90 mM for Na\(^+\) electrode; data not shown). In eight experiments, the slope of the \(V_{Na}\) vs. \(I_m\) (Fig. 6A) averages 100 ± 30 \(\Omega\). This is an apparent seal resistance since it is calculated using the total current of the oocyte instead of the current fraction passing through the membrane patch covered by the ISE. Assuming that the ISE covers 1/2,000th of the oocyte surface, this apparent resistance would correspond to an effective seal resistance of 1/2,000th of the oocyte surface, this apparent resistance would correspond to an effective seal resistance.
230 ± 70 kΩ. When this ohmic ΔV is subtracted from the ion-sensitive signal, a significant voltage-dependent Vion remains, which represents a real change in the local cationic concentration. This Vion is thought to represent the cations that bind to or are released from the cotransporter when a change in membrane potential produces a reorientation of the cation binding site. According to the current SGLT1 transport model, we expect an extracellular change in cation concentration that is proportional to the pre-steady-state charge transport model, we expect an extracellular change in cation transport activity at a different location on the oocyte surface (e.g., difference between animal and vegetal poles). This would probably be used to provide a qualitative description as the seal resistance and the trap volume are expected to change each time a given ISE is put in contact with the oocyte membrane.

In the presence of αMG, a slope in the VNa vs. time curve was observed. This slope can be used to estimate the equivalent height (hT) of the trap located between the oocyte plasma membrane invaginations and the ISE. The rate at which Na+ disappears from the trap (d[Na+]t/dt) during a hyperpolarizing voltage pulse can be estimated (a slope of −1.72 ± 0.30 mV/s at a Vm of −140 mV yields a d[Na+]t/dt of 665 ± 120 μM/s; see Fig. 5). As given in Eq. 2, this d[Na+]t/dt is equal to the change in oocyte Na+ flux (ΔItrigger between −50 and −140 mV) divided by the oocyte geometrical surface (S) × hT.

\[
\frac{d[Na^+]_T}{dt} = \frac{\Delta I_{Na}}{S \times h_T} = \frac{\Delta I_{Na}/F}{S \times h_T}
\]

where ΔItrigger is the change in the αMG-stimulated current (from −50 to −140 mV), and F is the Faraday constant. Interestingly, the area covered by the ISE cancels out from the calculation because a larger ISE would capture more of the whole oocyte Na+ flux but would also dilute these ions in a commensurately larger volume. As shown in Fig. 5, the cotransport current varies from −150 nA at −50 mV to −1,400 ± 100 nA at −140 mV. If we take 0.045 cm² as the geometrical surface area of a typical oocyte (radius ~600 μm), hT can be estimated at 4.9 ± 0.5 μm. As electron micrographs of oocytes show the presence of membrane invaginations in the first 10 μm (1) of the oocyte surface, this estimation of the average trap height appears quite reasonable.

Advantages and limitations of the ion-trap technique. The ion-trap technique is characterized by the combination of a large ISE and the presence of a constricted space where ion concentrations can increase or diminish. This combination allows for the detection of ion transport across membranes without having to rely on transmembrane current measurements or isotope flux measurements. The ion-trap technique has a much better time resolution than isotope flux measurement since changes in transport activities can be detected for an electrogenic transporters within 20 ms of the application of a voltage pulse. Compared with electrophysiology, the ion-trap technique has the advantage that, using steady-state measurements, it can be applied to electroneutral transport mechanisms, and the nature of the transported ion does not have to be determined by ion substitutions since the method is based on the direct detection of a transported ion. Extracellular measurements have previously been done with both static and vibrating probe methods (for examples, see Refs. 6, 14, 16, 17). With respect to vibrating probes, the ion-trap technique provides much better time resolution. With respect to static extracellular ISE, the advantage of the ion-trap technique relies on the presence of a microscopic detection volume (the trap), which provides better sensitivity as changes in ion transport will generate a larger change in ionic concentrations within this restricted space vs. the open extracellular space. Although not specifically employed as such in this study, the ion-trap technique could be used to study the distribution of a given transport activity at a different location on the oocyte surface (e.g., difference between animal and vegetal poles). This would probably be used to provide a qualitative description as the seal resistance and the trap volume are expected to change each time a given ISE is put in contact with the oocyte membrane.

One of the most serious disadvantages of the ion-trap technique is that the amplitude of the ion transport activity can only be determined relative to the transport measured in given basal condition. Since the ISE is pressed against the surface of an oocyte at resting potential, the ion flux through the membrane patch covered by the ISE is exactly balanced by the flux across the seal resistance between the ISE and the oocyte membrane. If a substrate is added, or if the Vm is changed, the ion-trap technique will only yield an estimation of the change that has occurred in the membrane ion flux with respect to the starting conditions.

Conclusion. We have presented a method that allows accurate measurement of ion transport activities without using isotopes or having to measure the transport current. The method is based on the measurement of ion capture and release in a reduced space between an ISE and the oocyte plasma membrane. The method presents good sensitivity and a time resolution that can reach 20 ms. Using the Na+/glucose co-transporter to test the ion-trap technique, we were able to demonstrate proportionality between the charge transferred and the surface rapid ΔVNa or ΔVNa. Moreover, there was direct proportionality between the cotransport currents and the slopes in the ISE signal. On a slower time scale (on the order of 20 s), the activity of the cotransporter can be detected without using its electrogentic properties, simply by adding the substrate. This demonstrates that the ion-trap technique can be applied to electroneutral ion transport as well. We predict that this method will be useful for testing transport mechanisms by looking at a different parameter than the traditional transport current or radioisotope uptake experiments.

GRANTS

This work was supported by the Canadian Institutes of Health Research Grant MOP-10580.

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