Video Article

Measurement of Ion Concentration in the Unstirred Boundary Layer with Open Patch-Clamp Pipette: Implications in Control of Ion Channels by Fluid Flow

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Abstract

Fluid flow is an important environmental stimulus that controls many physiological and pathological processes, such as fluid flow-induced vasodilation. Although the molecular mechanisms for the biological responses to fluid flow/shear force are not fully understood, fluid flow-mediated regulation of ion channel gating may contribute critically. Therefore, fluid flow/shear force sensitivity of ion channels has been studied using the patch-clamp technique. However, depending on the experimental protocol, the outcomes and interpretation of data can be erroneous. Here, we present experimental and theoretical evidence for fluid flow-related errors and provide methods for estimating, preventing, and correcting these errors. Changes in junction potential between the Ag/AgCl reference electrode and bathing fluid were measured with an open pipette filled with 3 M KCl. Fluid flow could then shift the liquid/metal junction potential to approximately 7 mV. Conversely, by measuring the voltage shift induced by fluid flow, we estimated the ion concentration in the unstirred boundary layer. In the static condition, the real ion concentrations adjacent to the Ag/AgCl reference electrode or ion channel inlet at the cell-membrane surface can reach as low as approximately 30% of that in the flow condition. Placing an agarose 3 M KCl bridge between the bathing fluid and reference electrode may have prevented this problem of junction potential shifting. However, the unstirred layer effect adjacent to the cell membrane surface could not be fixed in this way. Here, we provide a method for measuring real ion concentrations in the unstirred boundary layer with an open patch-clamp pipette, emphasizing the importance of using an agarose salt-bridge while studying fluid flow-induced regulation of ion currents. Therefore, this novel approach, which takes into consideration the real concentrations of ions in the unstirred boundary layer, may provide useful insight on the experimental design and data interpretation related to fluid shear stress regulation of ion channels.

Video Link

The video component of this article can be found at https://www.jove.com/video/58228/

Introduction

Fluid flow is an important environmental cue that controls many physiological and pathological processes such as fluid flow-induced vasodilation and fluid shear force-dependent vascular remodeling and development[1,2,3,4,5]. Although the molecular mechanisms for the biological responses to fluid flow shear force are not fully understood, it is believed that fluid flow-mediated regulation of ion channel gating may critically contribute to fluid flow-induced responses[5,6,7,8]. For example, activation of the endothelial inward rectifier Kir2.1 and Ca^{2+}-activated K^+ (K_{Ca}2.3, KCNN3) channels after Ca^{2+} influx by fluid flow has been suggested to contribute to fluid flow-induced vasodilation[6,7,8]. Therefore, many ion channels, especially mechanically-activated or -inhibited channels, have been studied in terms of fluid flow/shear force sensitivity with the patch-clamp technique[6,9,10,11]. However, depending on the experimental protocol performed during patch-clamp recording, outcomes and interpretation of the data on fluid flow-regulations of ion channels can be erroneous[10,11].

One source of fluid flow-induced artifacts in patch-clamp recording is from the junction potential between the bath fluid and Ag/AgCl reference electrode[11]. It is generally believed that the liquid/metal junction potential between the bathing fluid and Ag/AgCl electrode is constant as the Cl^- concentration of the bathing fluid is kept constant, considering the chemical response between the bathing solution and Ag/AgCl electrode to be:

Ag + $Cl^- \leftrightarrow$ AgCl + electron (e^-)   (**Equation 1**)

However, in a case where the overall electrochemical reaction between the bathing solution and Ag/AgCl reference electrode (**Equation 1**) proceeds from left to right, the Cl^- concentration of the bathing fluid adjacent to the Ag/AgCl reference electrode (unstirred boundary layer[12,13,14,15]) may be much lower than that in the bulk of bathing solution, unless enough convectional transport is ensured. Using an old or non-ideal Ag/AgCl electrode with inadequate chlorination of Ag may increase such a risk. This fluid flow-related artifact at the reference electrode, in fact, can be excluded by simply placing a conventional agarose-salt bridge between the bathing fluid and reference electrode, since the artifact is

based on alterations in real Cl⁻ concentration adjacent to the Ag/AgCl electrode[11]. The protocol presented in this study describes how to prevent the flow-related junction potential changes and measure real ion concentrations in the unstirred boundary layer.

After placing an agarose KCl bridge between the bathing fluid and Ag/AgCl reference electrode, there is another crucial factor that should be considered: just as the reference Ag/AgCl electrode acts like a Cl⁻ electrode, the ion channels also can function like an ion-selective electrode. The situation of an unstirred boundary layer between the bathing fluid and Ag/AgCl reference electrode arises during the movement of ions between the extracellular and intracellular solutions through the membrane ion channels. This implies that caution should be used when interpreting the regulation of ion channels by fluid flow. As discussed in our previous study[11], the movement of ions through a solution in which an electrochemical gradient is present can occur *via* three distinct mechanisms: diffusion, migration, and convection, where diffusion is the movement induced by concentration gradient, migration is the movement driven by electrical gradient, and convection is the movement through fluid-flow. Among these three transport mechanisms, convection mode contributes most to the movement of ions[11] (> 1,000 times greater than diffusion or migration under usual patch-clamp settings). This forms the theoretical basis of why junction potential between the bathing fluid and Ag/AgCl reference electrode can very under different static and fluid-flow conditions[11].

As per the hypothesis proposed above, some facilitatory effects of fluid flow on the ion channel current may be inferred from the convective restoration of real ion concentrations adjacent to the channel inlet at the membrane surface (unstirred boundary layer)[10]. In this case, the fluid flow-induced effects on ion channel currents have simply arisen from electrochemical events, not from the regulation of ion channel gating. A similar idea was previously suggested by Barry and colleagues[12,13,14,15] based on rigorous theoretical considerations and experimental evidence, also known as the unstirred layer or transport number effect. If some ion channels have sufficient single channel conductance and long enough open-time to provide sufficient transport rates through the channels (a faster transport rate in the membrane than in the unstirred membrane surface), a boundary layer effect may arise. Thus, the convection-dependent transport can contribute to the eventual fluid-flow-induced facilitations of ion current[10,12,13,14,15].

In this study, we emphasize the importance of using an agar or agarose salt-bridge while studying fluid-flow-induced regulation of ion currents. We also provide a method for measuring real ion concentrations in the unstirred boundary layer adjacent to the Ag/AgCl reference electrode and membrane ion channels. Furthermore, the theoretical interpretation of fluid flow-induced modulation of ion channel currents (*i.e.,* convection hypothesis or unstirred layer transport number effect) can provide valuable insights for designing and interpreting studies on the shear force-regulation of ion channels. According to the unstirred boundary layer transport number effect, we predict that ion channel currents through all types of membrane ion channels can be facilitated by fluid flow, independent of their biological sensitivity to fluid flow shear force, but only if the ion channels have sufficient single channel conductance and long open-time. Higher ion channel current densities may increase the unstirred boundary layer effect at the cell membrane surface.

Protocol

All experiments were performed in accordance with the institutional guidelines of Konkuk University.

1. Agarose Salt Bridges Between the Bath Solution and Ag/AgCl Reference Electrode

NOTE: Agarose 3M KCl salt bridges are produced as previously described[12] with minor variations.

1. Formation of bridges
    1. Bend the fire glass capillary tubes to form a U-shape as appropriate. The inner diameter of the capillaries should be large enough for reducing series resistance when recording large ion currents. Tubes with an inner diameter of 2-5 mm are usually acceptable.

2. Preparation of agarose 3 M KCl solution
    1. Prepare 100 mL of 3 M KCl solution (1 M or 2 M is also acceptable).
    2. Weigh 3 g of agarose.
    3. Dissolve the agarose in 100 mL of KCl (*i.e.,* 3% agarose) on a hot plate between 90 and 100 °C.

3. Loading the bridges with 3 M KCl agarose
    1. For easy loading, immerse the U-shaped glass bridges in the agarose-KCl solution.
        NOTE: It is easy to dig out the glass bridges if the agarose-KCl solution is contained in a shallow and broad container.
    2. Keep them overnight at room temperature (RT) for the agarose to set and harden.
    3. Carefully dig out the agarose-KCl-loaded glass bridges from the set/hardened agarose-salt.

4. Storing the bridges
    1. Prepare enough volume (*i.e.,* 500 mL) of the 3 M KCl solution in a wide-necked bottle.
    2. Store the prepared agarose-salt bridges in the bottle in a refrigerator.

2. Application of Fluid Flow Shear Force to Cells in a Patch-Clamping Chamber

NOTE: A schematic diagram of the patch-clamp experimental set-up is shown in **Figure 1**.

1. Place a container loaded with bathing solution (volume and height should already be measured) above the patch-clamp chamber.
2. Fill the patch-clamp chamber with the bathing solution by suctioning the tube.
3. To stop the fluid flow, clip the tube at the container's side to block the fluid flow, then clip the tube at the suction side to stop the suction at the same time. This is the "stationary" control condition.
4. To apply fluid flow shear force, open both tubes on the container and suction sides at the same time.

5. Before or after applying the fluid flow shear force to the cell, measure the flow rate in mL/min.
6. Calculate the flow rate by measuring the decrease in fluid volume over a given time.
7. From the measured flow rate and geometry (structure) of the bathing chamber, the shear force applied to the cell by the fluid flow should be estimated (see discussion section).
8. Alternatively, to control the flow rate (for steps 2.3-2.6), use a perfusion pump. In this case, be careful to ensure a constant rather than a pulsatile flow.

3. Measuring Changes in Liquid-Metal Junction Potential by Fluid Flow Between Bath Solution and Ag/AgCl Reference Electrode (Figure 3A)

1. Use the Ag/AgCl electrode or pellet, which is available from the ready-made products, without the agarose salt bridge.
2. Prepare a normal physiological salt saline for the bathing chamber (*e.g.*, 143 mM NaCl, 5.4 mM KCl, 0.33 mM NaH_2PO_4, 5 mM HEPES, 0.5 mM $MgCl_2$, 1.8 mM $CaCl_2$, 11 mM D-glucose; pH adjusted to 7.4 with NaOH).
3. Place a patch pipette containing a 3 M KCl solution in the chamber to minimize the junction potential shift between the pipette and bathing solutions.
4. Fix the voltage-clamp amplifier to the current clamp mode ("I = 0" or "CC").
5. After nullifying the initial offset potential, measure changes in voltage induced by various flow rates.
6. To verify that the changes in voltage are liquid/metal junction potentials, re-examine the effect of fluid flow on the junction potential using the agarose-salt bridge between the bath solution and Ag/AgCl electrode.

4. Experimental Estimation of Real Cl⁻ Concentration in the Unstirred Layer Adjacent to Ag/AgCl Electrode Under Static Condition (Figure 3B)

1. From the results of step 3, draw the junction potential-flow rate relationships and estimate the maximal (saturating) value of junction potential shift by the supra-fluid flow rate.
2. Prepare solutions with various concentrations of Cl (*i.e.,* 50, 99, 147, 195, and 288 mM of NaCl).
3. By changing the Cl⁻ concentration in the bathing fluid, draw the junction potential-[Cl⁻] relationship. Note that the fluid rate should be constant and sufficiently high (> 30 mL/min) to prevent the decrease of Cl⁻ concentration to that of the adjacent Ag/AgCl reference electrode.
4. From the two relationship curves, estimate the changes in Cl⁻ concentration from the measured junction potential shift.

Representative Results

Whole cell voltage-dependent L-type Ca^{2+} channel ($VDCC_L$) currents were recorded in the enzymatically dispersed rat mesenteric arterial myocytes, as previously described[11]. The arterial myocytes were dialyzed with a Cs-rich pipette solution under the nystatin-perforated configuration with divalent cation-free bathing solution to facilitate the current flow through $VDCC_L$[11,16]. Brief depolarizing voltage ramps or voltage steps, at a holding potential of -70 mV, were applied to elicit the $VDCC_L$ currents. A representative current-voltage (*I*-V) relationship in $VDCC_L$ in the absence and presence of fluid flow (5 mL/min or approximately 0.004 m/s), recorded with an agarose KCl bridge, is shown in **Figure 2A**. Fluid flow slightly increased the $VDCC_L$ current in a voltage-independent manner. This facilitating effect of fluid flow on the $VDCC_L$ current is summarized in **Figure 2B**.

The voltage-independent facilitation of $VDCC_L$ current by fluid flow is a proper response of the $VDCC_L$ to the fluid or shear force. The 5 mL/min or approximately 0.004 m/s of fluid flow in the current experimental setup was estimated to represent approximately 0.1 dyn/cm^2 in terms of shear force (see discussion). However, when the Ag/AgCl reference electrode was directly linked to the bathing fluid without an agarose KCl bridge, the *I*-V relationship in the presence of fluid flow shifted to the right compared to that of the $VDCC_L$ currents under a static condition (**Figure 2C** and **2D**). This resulted in the inhibition of $VDCC_L$ current at negative voltages and facilitation of $VDCC_L$ current at more depolarized or positive potentials. This exemplifies the fluid flow-induced artifact in patch-clamp recording in which a voltage shift of the *I*-V relation was not due to the modification of channel gating but was actually due to a junction potential shift between the bathing fluid and Ag/AgCl reference electrode[11]. Direct evidence for the fluid flow-induced junction potential shift is shown in **Figure 3**.

The junction potential shifts were measured according to step 3. The changes, due to fluid flow, were measured using an open pipette filled with 3 M KCl, as previously described[11]. With an open pipette filled with 3 M KCl, the junction potential between the pipette and bathing solutions could be minimized, and the potential changes due to fluid flow were primarily from the bathing solution and Ag/AgCl reference electrode. Without an agarose 3 M KCl bridge between the bathing fluid and Ag/AgCl reference electrode, fluid flow shifted the junction potential between the fluid and the Ag/AgCl electrode in a fluid flow rate-dependent manner (**Figure 3A**). The maximum junction potential change was extrapolated to be ~7 mV from the junction potential-fluid flow relationship (**Figure 3A**, bottom). In contrast, when the agarose 3 M KCl bridge was used, fluid flow did not alter the junction potential between the bathing fluid and reference electrode (summarized in the bottom graph of **Figure 3A**, bottom).

In order to measure concentration differences between the static and fluid flow conditions, in which enough convection modes of action are functional, we examined the effect of changing Cl^- concentrations on the bathing fluid-Ag/AgCl electrode junction potential according to step 4. Increasing the Cl^- concentration shifted the junction potential in a concentration-dependent manner (**Figure 3B**, top) just as fluid flow shifted the junction potential in a rate-dependent manner. Using a KCl agarose bridge, the junction potential was prevented from changing in a Cl^- concentration-dependent manner (**Figure 3C**), indicating that the junction potential change occurred between the bath solution and reference electrode, not between the bath and pipette solutions. The semi-log plot of the junction potential-[Cl^-] relationship is shown in the lower panel of **Figure 3B**. According to the results in **Figure 3B**, the extrapolated maximal value of ~7 mV in junction potential shift (from **Figure 3A**) suggests that the Cl^- concentration adjacent to the Ag/AgCl reference electrode decreases to ~70% of the average concentration of the bulk bathing fluid when fluid flow is absent (**Figure 3B**, bottom).

In our previous study, Kir2.1 currents were reported to be facilitated by fluid flow by convectively restoring (increasing) [K^+] at the channel inlet[10]. This idea stems from the phenomena occurring between the bathing fluid and Ag/AgCl electrode, as the Kir2.1 channel can function as a K^+ electrode just as the Ag/AgCl electrode functions as a Cl^- electrode. This idea is schematically illustrated in **Figures 4A** and **4B**. A representative example of fluid flow-induced facilitation of Kir2.1 currents is shown in **Figure 4C**. The Kir2.1 currents were elicited by a hyperpolarizing voltage step from a holding potential of 0 to -100 mV in rat basophilic leukemia (RBL) cells. Application of fluid flow (5 mL/min or 0.004 m/s) readily increased the Kir2.1 current (**Figure 4C**). This facilitation by fluid flow was previously suggested to be mediated not by cellular signaling but by the electrochemical effect of convective transportations of K^+ ions to the unstirred boundary layer[10].
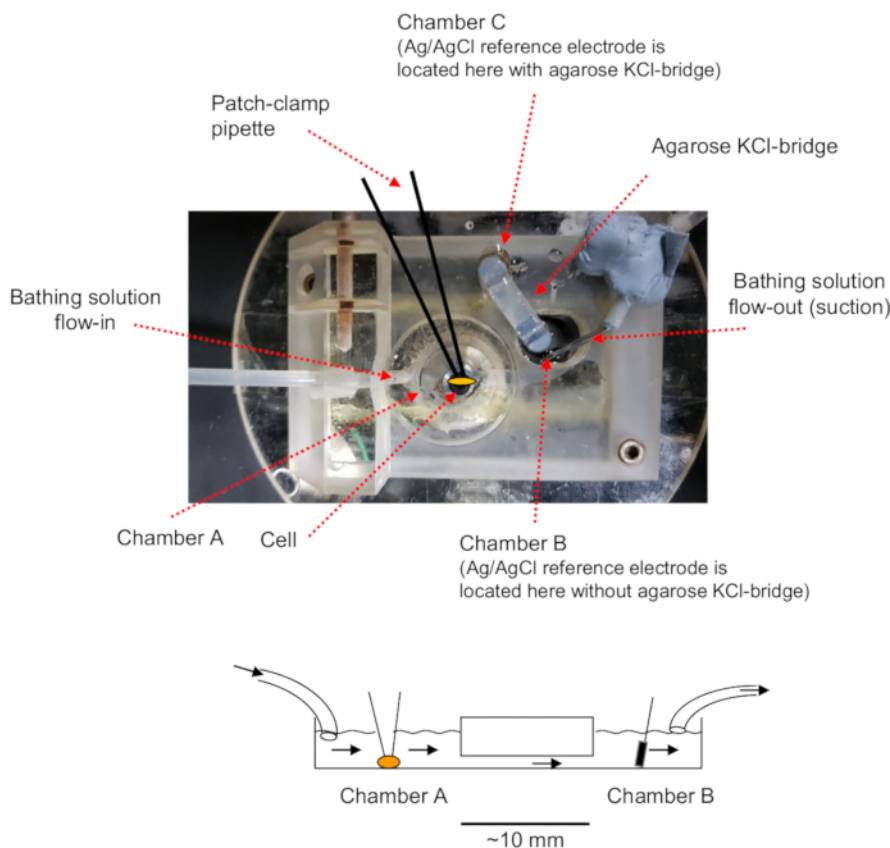

Figure 1: Schematic showing setup of the bathing chamber for the fluid-flow regulation of ion channels in the patch-clamp recording. Lower panel is the side view (sagittal section) of the patch-clamp chamber. It summarizes the path of fluid flow and locations of a studied cell, electrodes, and inlet/outlet of the fluid. Because the fluid is continuously pumped out through outlet tube by suction, the height of fluid in the chamber is maintained at a relatively constant level. This figure has been modified from a previous publication[11]. Please click here to view a larger version of this figure.
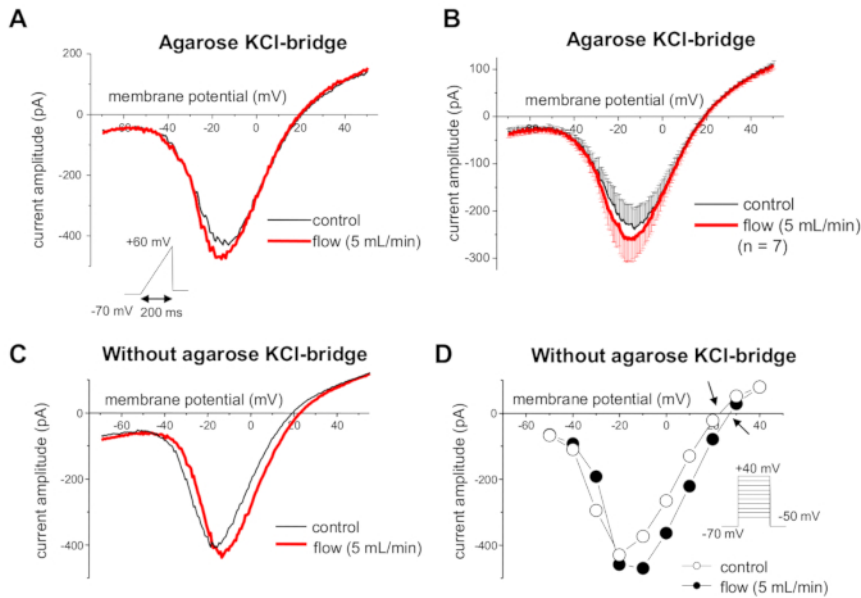
Figure 2: Effects of fluid flow on L-type voltage-dependent Ca^{2+} channel (VDCC$_L$) currents with and without the agarose 3 M KCl bridge. VDCC$_L$ currents were recorded in the enzymatically dispersed rat mesenteric arterial myocytes with nystatin perforated patch-clamp recording. Normal tyrode physiological salt solution with 4.2 mM EDTA without divalent cations was used as the bathing solution[11]. The pipette solution contained CsCl, 140 mM; MgCl$_2$, 1 mM; HEPES, 5 mM; EGTA 0.05 mM; pH adjusted to 7.2 with CsOH. (A and B) With agarose 3M KCl- bridge. (A) A representative *I*-V relationship for the VDCC$_L$ current and the effects of fluid flow. (B) Summary of the fluid effects on the *I*-V relationship of VDCC$_L$ currents. (C and D) Without agarose 3M KCl bridge. (C) *I*-V relationships of the VDCC$_L$ currents. (D) Summarized *I*-V relationships of the peak VDCC$_L$ currents in the absence and presence of fluid flow. The shapes of voltage steps for eliciting VDCC$_L$ currents are shown in the figure inset. This figure has been modified from a previous publication[11]. Please click here to view a larger version of this figure.
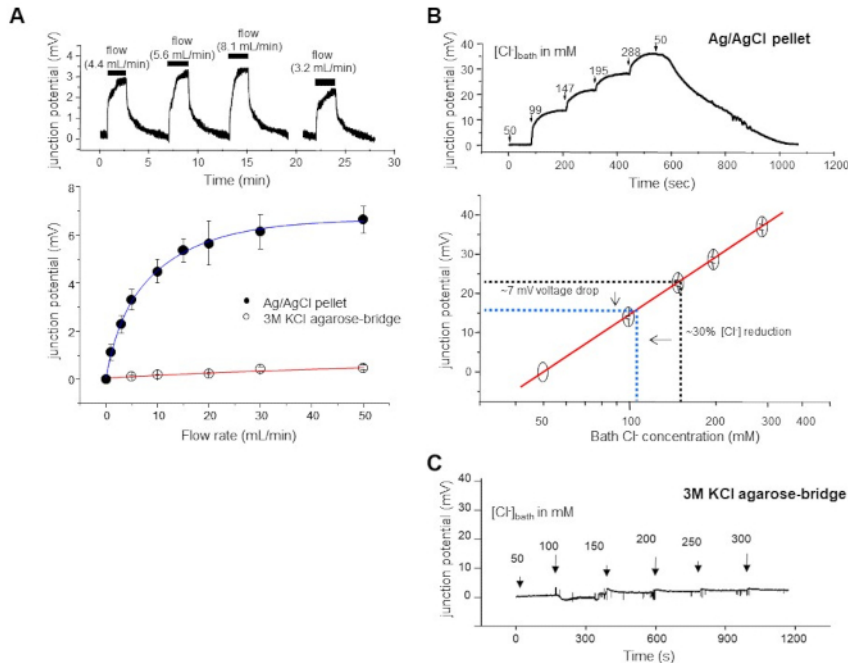
Figure 3: Effects of fluid flow on liquid-metal junction potential between the bathing fluid and Ag/AgCl reference electrode and estimation of real Cl⁻ concentration in the unstirred layer adjacent to the reference electrode from the measured junction potential.
(A) A representative tracing of junction potential changes due to various rates of fluid flow (upper panel). This figure has been modified from a previous publication[11]. The junction potential-fluid flow rate relationship (n = 5). (B) Upper panel:representative recording of junction potential changes due to various concentrations of NaCl solutions. Lower panel: the semi-log plot of the junction potential-[Cl⁻] relationship (n = 5). The straight line in red represents the best fit by a modified Nernst-equation for equilibrium potential with a ten-fold slope of 49 mV. Owing to the finite selectivity of Na⁺, compared to that of Cl⁻, for generating the liquid/metal junction potential, the slope value of 49 mV, instead of 58 mV, produced the best fit in the junction potential-[Cl⁻] relation at room temperature. The 49-mV slope indicates the Cl⁻ dependence (or selectivity) of the Ag/AgCl reference electrode > 95% over the other ion (in this case, Na⁺), according to the Goldman-Hodgkin-Katz voltage equation. A shift of 7 mV at an Cl⁻ concentration of 150 mM indicates a decrease of ~30% in the Cl⁻ concentration. (C) A representative tracing of junction potential in various concentrations of NaCl solutions with a use of 3 M KCl agarose bridge (n = 3). Please click here to view a larger version of this figure.
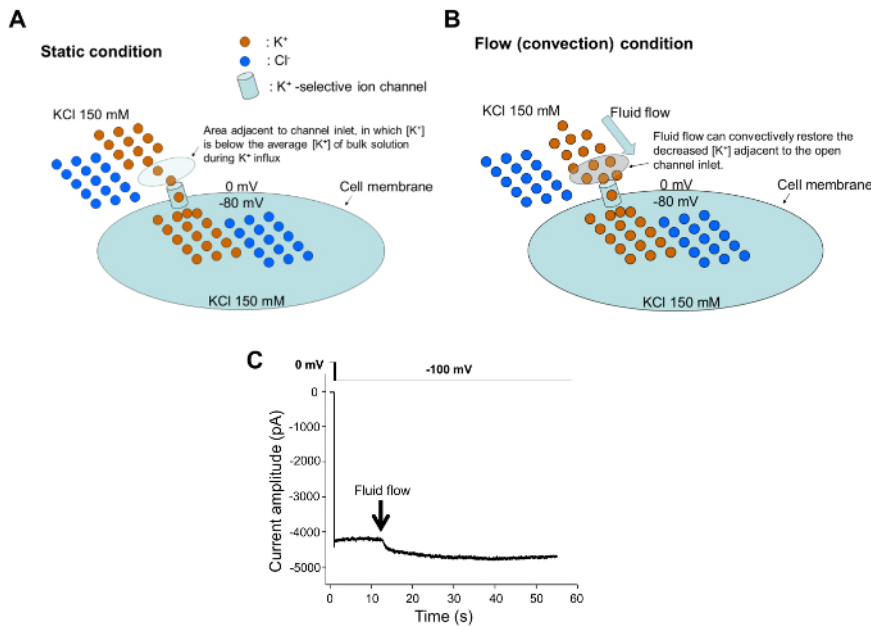
Figure 4: Schematic of the effects of convection model of fluid flow on the ion concentrations adjacent to the open channels during ion current flux. (A) Under static conditions with little convective transport of ions in the solution with electric field, the K^{+} ion flux through K^{+}-selective ion channels can cause a decrease in K^{+} concentrations in the microdomain adjacent to the channel inlet. (B) Fluid flow can convectively restore the decrease in K^{+} concentration adjacent to the open channel inlet. (C) Effect of fluid flow on the inward rectifier Kir2.1 channel currents. Fluid flow instantly increased the Kir2.1 currents. The shape of the voltage step is shown in the figure inset. The Kir2.1 currents were recorded using high K^{+}-bathing and -pipette solutions. Bathing solution: 148.4 mM KCl, 0.33 mM NaH_2PO_4, 5 mM HEPES, 0.5 mM $MgCl_2$, 1.8 mM $CaCl_2$, 11 mM D-glucose; pH adjusted to 7.4 with NaOH. Pipette solution: 135 mM KCl, 5 mM NaCl, 5 mM Mg-ATP, 10 mM HEPES, 5 mM ethyleneglycol-bis (2-aminoethyl)-N,N,N',N',-tetraacetic acid (EGTA), pH 7.2 (adjusted with KOH). Since RBL-2H3 cells are highly susceptible to hypo-osmotic swelling and consequent trigger of volume-activated Cl^{-} currents, 38 mM sucrose was added to the bathing solution to adjust for osmolarity and prevent cell swelling. Moreover, a Cl^{-} channel blocker [4,4'-diisothiocyano-2,2'-stilbenedisulfonic acid (DIDS, 30 µM)] was added to the pipette solution to eliminate any contamination by Cl^{-} currents. Panel C has been modified from a previous publication[10]. Please click here to view a larger version of this figure.

Discussion

In this study, we demonstrated a method to measure real Cl^{-} concentration in the unstirred layer adjacent to the Ag/AgCl reference electrode by determining the liquid-metal junction potential with an open patch-clamp pipette filled with a high KCl concentration. The change in Cl^{-} concentration in the boundary layer can result in a shift of junction potential when switching from static to fluid-flow conditions. Simply using an agarose KCl bridge between the reference electrode and bathing fluid can prevent the Cl^{-} concentration-related errors or artifacts during patch-clamp recording.

Besides emphasizing the importance of an agar or agarose salt bridge, another application of this method in estimating real ion concentration in the unstirred boundary layer is as follows. Because plasmalemmal ion channels can function as ion-selective electrodes (just as the Ag/AgCl electrode functions like a Cl^{-} electrode), the real ion concentration in the unstirred boundary layer adjacent to the channel inlet at the cell membrane surface can be different from the average concentration of the bulk fluid. This difference in ion concentration between the bulk fluid and unstirred layer adjacent to cell membrane is the real scenario under clinical settings and should be distinguished from the biological modulation of channel gating by fluid flow/shear force. Unfortunately, unlike the unstirred layer effect between the Ag/AgCl reference electrode and bathing fluid, we cannot fix the unstirred layer effect adjacent to the cell membrane surface when studying the regulation of ion channels by fluid flow/shear force.

However, considering the observation that real ion concentration in the unstirred layer is approximately 70% of that in bulk fluid (**Figure 3**), we can make some amendments in the experimental data to distinguish the biological modulation of ion channels from the "electrochemical phenomenon of unstirred layer effect". The real ion concentration in the unstirred layer at the cell membrane surface was expected to be approximately 70% of the average concentration of the bulk bathing solution in a recent study[10]. Since fluid flow restored the decreased ion concentration, it facilitated the Kir2.1 current independently of cellular signaling[10]. In our previous study, current density was considerably high (2.5 A/m^{2}) with a high extracellular K^{+} concentration and high expression of Kir2.1 in RBL cells[10]. However, in the case of real cell membranes with various ion channel current density amplitudes, the unstirred layer effect at the cell membrane surface may depend greatly on the amplitude of ion channel current density. Besides, this may cause some ion channel currents (especially those with relatively lower current densities) to be insensitive to fluid-flow regulation; although, the unstirred layer effect is regulated electrochemically and not biologically. Thus, this may affect the technique described here. Therefore, the possibility of developing a quantitative method that is adequate for correcting experimental results should be investigated in future studies.

In **Figure 3**, we observed that liquid-metal junction potential between the Ag/AgCl reference electrode and bathing fluid was greatly dependent on the condition of the Ag/AgCl electrode. In fact, when the Ag/AgCl electrode was perfect in condition, changes in junction potential due to fluid

flow was minimal (data not shown). However, poor chlorination of the Ag/AgCl electrode caused a greater shift in the junction potential. Since the Ag/AgCl reference electrode is very susceptible to various external stimuli, such as ultraviolet light and oxidative stress, using an agar or agarose KCl bridge is always recommended. Although changes in junction potential by fluid flow between bathing fluid and the reference electrode is a potential source of error, we successfully estimated the real ion concentrations in the unstirred boundary layer by measuring the shift of junction potential under various fluid-flow rates (**Figure 3A** and **3B**).

The critical point in step 4 for preparing the standard curve for the estimation of real Cl^- concentration in the unstirred boundary layers from the shift of junction potential is that the standard curve should be recorded under a sufficent flow rate (30 mL/min in this experiment). Although this flow rate is very fast, in practical cases the faster the fluid, the smaller the concentration drop is at the boundary layers (**Figure 3**). In addition, the open pipette must be filled with high KCl, instead of a regular pipette solution, in order for a patch-clamp study to prevent the change in junction potential between a pipette and bathing solution.

The shear force in the patch-clamp setting can be estimated from the following relationship[11]:

$$\tau = (6\mu Q) / (bh^2) \qquad \textbf{(Equation 2)}$$

Where: τ is the shear stress (N/cm^2); μ is the viscosity ($0.001\ N\ m/s^2$ for water at 20°C); Q is the fluid flow rate (m^3/s); b is the chamber width (m); and h is the chamber height (m). When the fluid flow rate is 30 mL/min, the shear force in the patch-chamber shown in **Figure 1** is estimated to be ~0.75 dyn/cm^2 according to the above equation. This is a low shear force level compared to the physiological shear force; endothelial cells in blood vessels can be subjected to shear forces of up to 40 dyn/cm^2[18,19]. Therefore, provided that the ion channels are not sensitive to shear forces less than 0.75 dyn/cm^2, we can study the fluid flow/shear force sensitivity of ion channels after excluding the unstirred boundary layer effect by setting the control condition to 0.75 dyn/cm^2. However, some ion channels, including Kir2.1, seem to be sensitive to shear forces less than 0.75 dyn/cm^2[2,3,4,5,6].

The unstirred layer effect was originally suggested by Barry and colleagues[12,13,14,15]. Here, we provide a method to estimate real ion concentration in the unstirred layer by measuring changes in junction potential with open patch-clamp pipette. We also suggest that this unstirred boundary layer effect may contribute to fluid flow-induced regulation of ion channel currents and should be considered while studying fluid flow-mechanosensitivity of ion channels. However, based on this hypothesis, it may be asked why some ion channel currents are not sensitive to fluid flow-dependent regulation if the unstirred boundary layer effect is an electrochemical rather than biological control. As briefly addressed above, this is probably because only ion currents through channels with large enough single-channel conductance and long enough open-time can be facilitated by fluid flow. That is, for the establishment of the unstirred layer in which the ion concentration is different from the average in bulk solution, flux in the membrane phase should be rapid enough compared to that in the aqueous phase[14]. We have recently suggested that the current through Kir2.1 channels, whose conductance and open time are sufficiently high, is facilitated by fluid flow *via* mechanisms of convective restoration of ion concentration in the unstirred boundary layer of cell membrane surface[11].

In conclusion, we present a method for measuring ion concentration in the unstirred boundary layer adjacent to the reference electrode and cell membrane surface with an open patch-clamp pipette. Besides emphasizing the importance of an agarose KCl bridge, this method also provides a way to account for the unstirred layer effect while interpreting fluid flow/shear force control of ion channels.

Disclosures

The authors have nothing to disclose.

Acknowledgements

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