

Rapid Solution Exchange for Recording of Fast-Desensitizing Ligand-Gated Ion Channels on the IonWorks Barracuda System

By *Xin Jiang, Ph.D., Jane Hinton, Ph.D. Karen Cook, M.S., Jeff Netzeband, Ph.D. and James L. Costantin, Ph.D., Molecular Devices, Inc. 1311 Orleans Drive, Sunnyvale, CA 94089.*

Introduction

Ligand-gated ion channels (LGICs) mediate fast synaptic transmission in the nervous system and the neuromuscular junction. Gated by the binding of neurotransmitters to the ion channels, LGICs transduce chemical signals into electrical signals, *i.e.*, an ion influx into the postsynaptic cell. After activation, many LGICs quickly enter a desensitized state, often within milliseconds, in which the neurotransmitter remains bound but the ion channel is closed. The transient nature of the LGIC activation and subsequent desensitization is crucial for fast synaptic signaling. Consequently, the measurement of LGIC activities requires the ability to change solutions rapidly, so to outpace the desensitization of the ion channel and secondly, accurately control the concentration of compound reaching the ligand binding sites.

The IonWorks Barracuda™ Automated Patch Clamp System combines 384 parallel patch clamp amplifiers and a 384-channel pipettor head for the recording of ionic currents in 384 wells simultaneously. The assay plate (PatchPlate™ Consumable) features a uniquely designed “flow-through” configuration that is optimized for rapid solution exchange. This is especially important when measuring fast LGICs in order to resolve the rapid activation and desensitization of the ionic current kinetics. To quantify the solution exchange rates on the IonWorks Barracuda System, in this study we measured the real-time change of $K_v1.3$ current in voltage-clamped cells after the regular external buffer was replaced by buffer containing high potassium (high K^+). The experimental design removes any acceleration of current kinetics caused by ionic current passing through partially activated channels as would be the case with partially activated LGICs. The $K_v1.3$ channel is opened by voltage prior to the addition of high K^+ so the change in current by high K^+ is dictated purely by the driving force for K^+ . We found that the decay-time for the resultant change of current is as short as 39 ms, indicating that the IonWorks Barracuda System enables ultra-fast solution exchange. We also present successful recordings of $\alpha 7$ -nAChR and $\alpha 1$ -nAChR currents, demonstrating that the IonWorks Barracuda System can reliably record rapidly desensitizing LGICs.

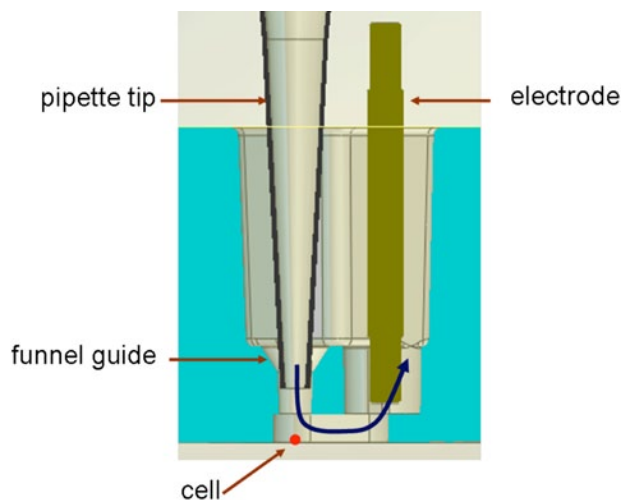
Methods and Results

Chinese hamster ovary (CHO) cells stably transfected with human $\alpha 7$ -nicotinic acetylcholine receptor (nAChR) were provided by ChanTest® Corporation (Cleveland, OH). TE-671 cells endogenously expressing $\alpha 1$ - nAChR were obtained from ATCC. The stably transfected $K_v1.3$ ion channel cell line was developed at Molecular Devices, Inc. For electrophysiological recordings on the IonWorks Barracuda System the currents were elicited by 500 μM of acetylcholine (Ach).

Unique "flow-through" design of PatchPlate for rapid solution exchange

Each well of the PatchPlate Consumable has on one side the entry point for a dedicated silver-chloride recording electrode and on the opposite side a dedicated pipette tip. The two sides are connected by a small chamber where cells reside; and by a common chamber at the top above the Teflon coating of the electrode (Figure 1). This unique "flow-through" design is crucial for fast solution exchange as the stream of compound dispensed from the pipette tip quickly replaces the solution over the recorded cell(s).

The cross-section diagram of an individual PatchPlate Consumable well (Figure 1)

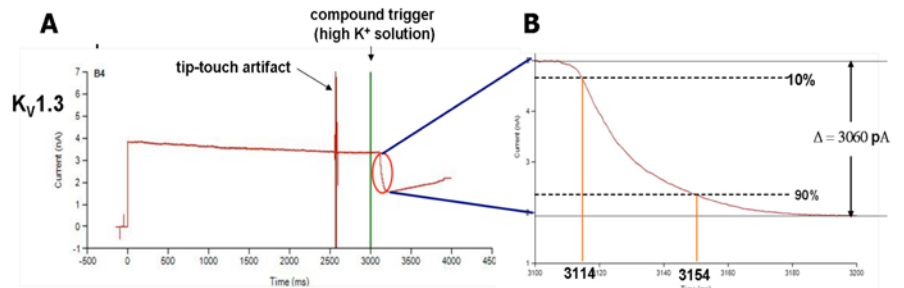


The pipette tip and recording electrode co-exist in the same well for simultaneous compound addition and data acquisition, which is necessary for real-time measurement of LGIC currents. The funnel guide precisely locates the pipette tip above the cell to ensure it is consistently positioned in each well of the Patch Plate consumable. The blue arrow indicates the movement of solution in a "flow-through" configuration. The cells are placed in the small chamber on the bottom, directly below the pipette tip, for fast and efficient solution exchange.

Measurement of solution exchange rate of the IonWorks Barracuda System

In this study, the rate of solution exchange of the IonWorks Barracuda System was tested by using $K_v1.3$ ion channel stably expressed in CHO cell. When subjected to a depolarizing voltage step to +20 mV, the $K_v1.3$ ion channel was quickly driven into the open state and the resultant outward current was recorded continuously. Due to slow channel inactivation ($\tau = \sim 300$ ms at 20 mV), the amplitude of current remained relatively stable (Figure 2). Changing external buffer from low (4.1 mM) to high (70 mM) concentration of K^+ reduced the driving force for potassium which leads to the reduction of $K_v1.3$ current. Since the ion channel remains open over the course of recording, there is no gating involved and the time course for the change of current reflects solely the rate of solution exchange.

Experimental procedure to determine the solution exchange rate (Figure 2)



Example recording of $K_v1.3$ current illustrating the measurement of solution exchange rate. A) $K_v1.3$ current is elicited by a 4s depolarizing step to +20 mV ($V_h = -70$ mV); the initial external buffer (D-PBS) contains 4.1 mM of K^+ . Three seconds into the protocol, high K^+ buffer (70 mM) was applied onto the cells. The following reduction of the current magnitude reflects purely the reduction of K^+ driving force in response to solution exchange (red circle). B) Zoom in view of the circled area in A); the solution exchange rate, measured by 10-90% rise time, is 40 ms for this cell.

The solution exchange rate under different experimental conditions

Based on fluid mechanics, the primary factors controlling the rate of solution exchange in a cell perfusion system are the flow velocity, proximity of the cell to the exit ports, and the surface area of the cell. To provide maximal flexibility for different assays, the IonWorks Barracuda System allows multiple settings for both the height of the pipettor tip (high, medium and low relative to the bottom of the chamber); and the dispensing speed. In addition, the apparent solution exchange rate in the single-hole (SH) mode is faster than the population patch clamp (PPC) mode, presumably because the cell surface area in the PPC mode is larger (Table 1) and spread over a larger area of the substrate.

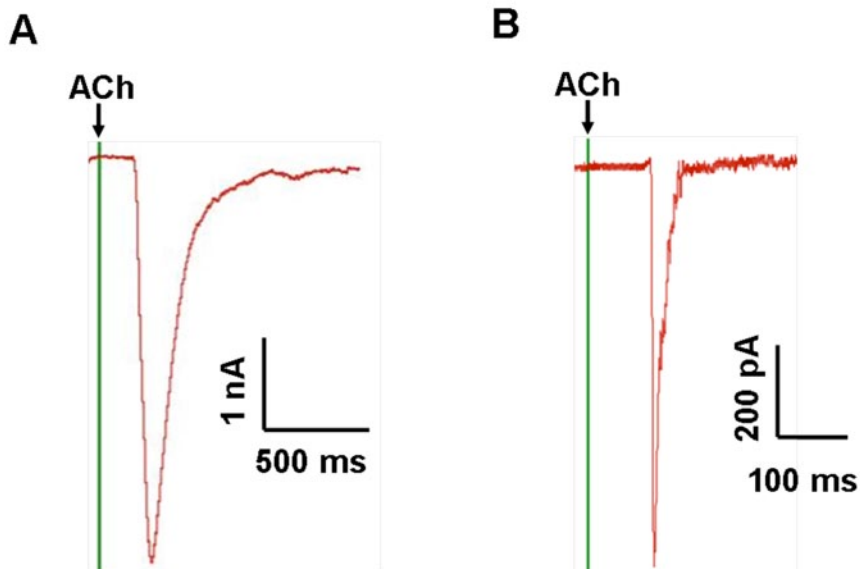
Recording mode	Pipettor height (low), dispensing speed (20 μ L/s)	Pipettor height (medium) dispensing speed (10 μ L/s)
SH	39 \pm 6	164 \pm 19
PPC	77 \pm 12	254 \pm 29

Note: The pipettor tip is ~ 0.2 mm from the cell at "low" height; and ~ 1.0 mm at "medium" height. All values represent mean \pm SD ($n = 8$ replicates / condition).

Recording of fast-desensitizing LGICs on the IonWorks Barracuda System

The rapid solution exchange rate of the IonWorks Barracuda System makes it possible to record even ultra-fast desensitizing ion channel such as nicotinic acetylcholine receptors. As shown in Figure 3, $\alpha 7$ -nAChR, which completely inactivates in ~ 30 ms, and $\alpha 1$ -nAChR, which inactivates in ~ 1 s, can be faithfully recorded.

Fast-desensitizing LGICs on the IonWorks Barracuda System (Figure 3)



Recordings of fast-desensitizing LGICs on the IonWorks Barracuda System. A) SH recording of $\alpha 1$ -nAChR current elicited by 500 μ M of acetylcholine ($V_h = -70$ mV). For this recording, the 10-90% time to peak current is 21 ms. B) SH recording of $\alpha 7$ -nAChR current elicited by 500 μ M of acetylcholine ($V_h = -70$ mV). For this recording, the 10-90% time to peak current is 5.4 ms.

Summary

Rapid solution exchange is crucial for evaluating LGICs due to their characteristic desensitization following activation. In this study we used $K_v 1.3$ ion channel as the model and measured the solution exchange rate on the IonWorks Barracuda System. The data demonstrate that the fluid exchange on the IonWorks Barracuda System is fast and complete, making it applicable for analyzing even rapidly desensitizing LGIC targets such as $\alpha 7$ -nAChR. The capability for the IonWorks Barracuda System to rapidly exchange solutions, together with its 384 parallel recording sites for the generation of > 1,100 data points per hour, provides a perfect solution to the unmet demand of high-throughput, low-cost, electrophysiological assays for rapidly desensitizing LGICs.

References

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2. Papke RL and Porter Papke JK. (2002) Comparative pharmacology of rat and human $\alpha 7$ nAChR conducted with net charge analysis. *Br J Pharmacol* 137(1):49-61.



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