

Rapid Solution Exchange and Ligand-Gated Channel Studies on the PatchXpress 7000A Automated Patch Clamp System

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Abstract

The PatchXpress® 7000A patch clamp system is an automated electrophysiology workstation that allows users to increase throughput of research quality recordings and to measure ion channel activity from nearly any channel type. The unique *SealChip™* planar electrodes by AVIVA Biosciences and the PatchXpress fluidics system support high quality patch-clamp recordings of both voltage-gated and ligand-gated ion channels. The system uses a robotic pipettor to deliver ligand and drugs directly to the patched cells in the whole-cell configuration. Washout of ligand in each well is achieved by employing a pair of dedicated perfusion tips in close proximity to the base of the recording well for rapid fluid exchange near the cell. In order to quantify the speed of ligand and drug addition, we studied fluid exchange rates on the PatchXpress 7000A system using RBL cells endogenously-expressing inward-rectifier K channels and changed the concentration of external potassium [K⁺]. Going from low to high [K⁺] increased the current with an exponential time course and a single time constant of 19±11 ms. Washing out the high [K⁺] with the independent wash station has a time constant of 420±281 ms.

We further tested the system with cells expressing ligand-gated channels, including HEK cells endogenously expressing pH-sensitive ASIC channels as well as L-tk cells heterologously-expressing GABA_A receptors. A number of ligands were tested successfully on both cell/channel types. The interaction of ligand and specific agonists and antagonists with the channels was recorded. Complete washout of ligand (GABA) is easily obtained with the independent wash station. Our results demonstrate that the properties of the fluid delivery system together with a newly added software feature for precise timing of ligand and drug delivery, make the PatchXpress 7000A platform suitable for a wide range of ligand-gated ion channel studies.



Figure 1. PatchXpress 7000A Automated Patch Clamp System.

- Key features of the system:
- 16 channel parallel amplifiers
 - 16 channel parallel fluidics
 - True gigaseals
 - Whole-cell recording
 - Access resistance compensation
 - Capacitance compensation
 - Disposable pipette tips

16-Channel Wash Station



Figure 2. PatchXpress fluidics system. Photograph of the pipetting robot and 16-channel wash station.

Separate wash station perfuses each cell independently with buffer to wash out compound and/or ligand between additions and frees the pipetting robot to be used only for drug or ligand addition and not for washes. The independent wash station makes it possible to remove excess cells and to wash cells for as long as it takes to remove drug or ligand.

Experimental conditions

Cell lines:

RBL cells endogenously expressing inward rectifier potassium channel
L-tk cells expressing GABA_A receptor (α,β_{2γ})
HEK 293 cells endogenously expressing ASIC channel

Hardware: PatchXpress 7000A
Software: PatchXpress commander 1.6 (beta)

System setting:

Pipette delivery speed: 100 ul/s
Suction during addition: ON
Wash station tip Z offset: 2.0 mm
Wash station tip X offset: -2.0 mm
Wash station height from substrate: 1.0 mm
Lock robot function (new to Ver1.6): ON

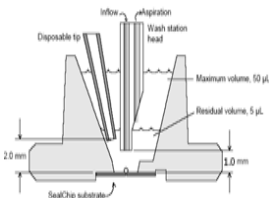


Figure 3. Schematics of the relationships among the cell on the substrate, disposable pipette tip, orifices of inflow and outflow of the wash station.

Rapid Solution Exchange

The rate of fluid exchange of PatchXpress 7000A was tested by using RBL cells endogenously expressing inward rectifier potassium channels and changed the concentration of external potassium.

RBL cells were held at -120 mV. Inward rectifier channel activity was recorded as continuous inward leak currents. Changing external buffer from low to high concentration of potassium increased the leak current. Washing out the high [K⁺] solution restore current to baseline level.

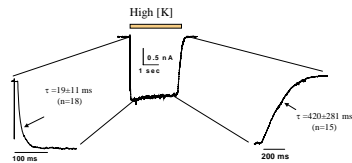


Fig 4. Solution exchange rate High [K⁺] solution induce a current change with an single exponential time course and a time constant of 19 ± 11 ms. Washing out the high [K⁺] with the independent wash stations has a time constant of 420 ± 281 ms.

GABA_A receptor ligand action

L-tk cells expressing recombinant GABA_A receptors (α,β_{2γ}) have been tested. γ-Aminobutyric acid (GABA) elicited inward chloride currents at a holding potential of -60 mV. GABA action can be completely washed out. Therefore, repetitive applications of ligand have been used.

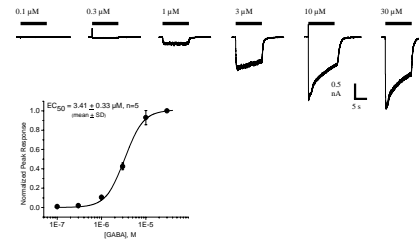


Fig 5. GABA induced currents and GABA EC₅₀. Top: six increasing concentrations of GABA were applied as indicated by the bars, including a saturating concentration of GABA (30 µM). Bottom: the mean concentration-response relationship to GABA for cells expressing GABA_A receptors with a mean EC₅₀ of 3.41 ± 0.33 µM. (mean ± S.D., n=5).

GABA_A receptor pharmacology

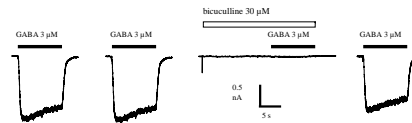


Fig 6. Antagonist action GABA-induced currents were consistently repeated by 3 µM of GABA and inhibited by the competitive antagonist bicuculline. L-tk cells were pre-incubated with bicuculline for 15 seconds before co-application with 3 µM GABA. The pre-incubation period was identical for all cells by using the "lock the compound robot" feature of the PatchXpress commander software (new in Ver 1.6). This feature ensures to have precise timing across cells to generate the consistent results.

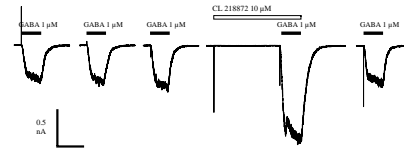


Fig 7. Benzodiazepine agonist action Benzodiazepines (BZD) ligands allosterically potentiate GABA currents by increasing the GABA-gated Cl⁻ conductance. Figure shows the highly reproducible responses (three applications with wash out in between) of GABA-induced currents recorded by PatchXpress 7000A and the effect of CL218872 to potentiate GABA-mediated currents.

Acid-Sensing Ion Channel (ASIC)

ASIC channel endogenously expressed in HEK 293 cells have also been tested on PatchXpress 7000A. Cationic currents were elicited by changing extracellular pH from 7.4 to 4.4. Amiloride, an ASIC channel blocker was applied to demonstrate the inhibitory effect.

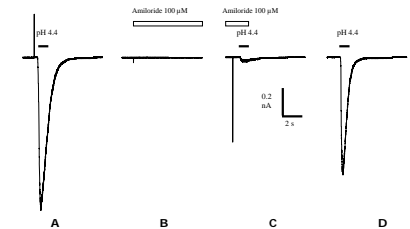


Fig 8. ASIC channel recording. Rapid desensitizing proton induced currents. Cells were held at -60 mV. From left to right: A) pH4.4 induces transient inward current; B) Amiloride alone has no effect on the cell; C) Amiloride blocks pH4.4 induced current; D) After washing out amiloride and low pH buffer, pH sensitive current restored.

Summary:

- Multiple types of ligand-gated ion channels have been tested on PatchXpress 7000A system.
- Fluid exchange on PatchXpress 7000A is fast and reliable. Ligand delivery shows a single exponential time course.
- Ligand delivery time constant is 19 ± 11 ms. Wash off time constant is 420 ± 281 ms.
- Complete wash out of ligand/drug can be achieved.
- A single cell can be used to generate EC₅₀.
- The new function of "Lock the compound robot" in software version 1.6 is capable of precisely control the timing of the ligand/drug delivery.

Acknowledgements:

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