Dynaflow™ 48, a microfluidic chip solution for increasing throughput and data quality in patch-clamp-based drug screening*

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Abstract

Ion channels are transmembrane proteins, found in virtually all cell types throughout the human body. Ion channels underlie neural communication, memory, behavior, every movement and heartbeat, and are as such prone to cause disease if malfunctioning. Therefore ion channels are very important targets in drug discovery. The gold standard technique for obtaining information on ion channel function with high information content and temporal resolution is patch-clamp. The technique measures the minute currents originating from the movement of ions across the cellular membrane, and enables determination of the potency and efficacy of a drug. However, patch-clamp suffers from serious throughput restrictions due to its laborious nature. To address the throughput problems we have developed a microfluidic chip containing 48 microchannels for an extremely rapid, sequential delivery of a large number of completely controlled solution environments to a lifted, patch-clamped cell. In this way, throughput is increased drastically compared to classical patch-clamp perfusion set-ups, with uncompromised data quality. The 48-microchannel chip has been used for the characterization of drugs affecting ligand-gated ion channels including agonists, antagonists and positive modulators with positive effects on both throughput and data quality.

Keywords: high throughput, ion channel screening, patch clamp, microfluidics, $GABA_A$ receptor

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* We acknowledge the support of professor Owe Orwar. This work was founded by the Göran Gustafsson Foundation and the Swedish Foundation for Strategic Research (SSF).

Introduction

Ion channels are of central importance in the body. They serve as conduits between the inside of cells and the outside world, controlling basic bodily processes such as heartbeats, metabolism and immunity as well as more complex processes such as emotions, learning and memory (1). Increasing evidence indicates that the direct or indirect malfunctioning of an ion channel can severely affect human health and many ion channels are thus considered to be important therapeutic targets (2). The standard technique for acquiring high-information content functional ion channel data is the patch clamp method (3). The technique relies on the electric clamping of the cellular membrane potential of a single cell, while measuring the ionic current caused by ion channel activity. Due to the high temporal resolution and the sensitivity of the technique, it is possible to record the activity of single ion channels with sub-millisecond time resolution. However, acquiring high quality patch clamp data is a tedious process, requiring extensive work input from highly skilled operators. Due to these limitations, the identification of compounds that affect the desired ion channel function is cumbersome and time-consuming. As the need for high quality ion channel data continues to increase, the patch clamp technique has become the bottleneck in the efforts to increase productivity in the process of ion channel screening. Therefore, large efforts have been made in developing parallel patch clamp devices that aim to automate and multiplex the number of patch-clamped cells and thereby increase throughput in screening applications (4-10). Although the automated patch clamp devices indeed offer an increased rate of compound testing, this improvement unfortunately comes at a price, being reduced recording quality, time resolution and loss of flexibility and control during the experiment (11). In particular, the automated patch clamp systems are not amenable for screening ligand-gated ion channel targets, mainly because these systems lack the ability for rapid and precise solution switching. Because activation and desensitization of many types of ligand-gated ion-channels occurs within a few milliseconds (1,12-14), methods are required that can change a large number of solutions containing different ligands around cells in the same time range in order to efficiently study such rapid receptor interactions. Examples of pharmacologically important ion channels that display fast kinetics are nicotinic acethylcholine receptors, controlling muscle function, and ligand-gated channels of fast chemical synapses such as GABA and AMPA receptors, involved in neuronal modulation. Consequently, the need for development of screening methods for ion channels that offer high throughput, low cost, fast, flexible and well-controlled solution exchange while still maintaining the high

information content offered by classic patch clamp screening is growing at a rapid pace.

Rather than resorting to a parallel patch-clamp concept, we have developed a system where conventional single-cell-based patch clamp technique is combined with chip-based microfluidics for rapid sequential delivery of ion channel agonists or antagonists onto patch-clamped cells (15,16). Here, we extend the previous findings and demonstrate a 48 channel chip facilitating advanced investigations of ion channel function by utilization of complex substance application patterns, possibly involving a range of different substances to be applied to the cell sequentially. To demonstrate the functionality of this device we performed patch clamp recordings of several different dose response curves from a single cell on one chip. Furthermore, we demonstrate that the excellent experimental control, and solution switching properties, enables direct extraction of kinetic parameters from the obtained data sets.

Materials and Methods

Cell culture. The WSS-1 cell line (HEK 293 transfected with $GABA_A$ receptor) was used. Cells were grown adherent for 2-4 days on plastic Petri dishes in DMEM supplemented with antibiotics, L-glutamine, glucose (4.5g/L), Na-carbonate and fetal calf serum (10%).

Solutions and drugs. The extracellular solution used in all experiments contained, in mM; 140 NaCl, 5 KCl, 1 $MgCl_2$, 1 $CaCl_2$, 10 Hepes, 10 D-Glucose, pH 7,4. The electrodes were filled with intracellular solution, in mM; 100 KCl, 2 $MgCl_2$, 1 $CaCl_2$, 11 EGTA, 10 Hepes, pH 7.2. GABA was obtained from Calbiochem (La Jolla, CA) and β-alanine and bicuculline methiodide, were from Sigma (St. Louis, MO).

Electrophysiology. Whole cell patch clamp methods were used in all experiments. Glass electrodes were fabricated from thick wall borosilicate glass and the resistances of the pulled patch pipettes were 3-5 MΩ. All data was recorded using an Axopatch2B (Axon Instruments) patch clamp amplifier. The cells were clamped at -40mV. Current signals were recorded at a sampling frequency of 5 kHz, low pass filtered (1 kHz, 4-pole Bessel filter) and analyzed using the Clampfit8 software. Two different GABA receptor agonists (GABA and β-alanine) and one antagonist (Bicuculline) were used. Every other well was loaded with buffer for washing interdigitated with the various concentrations of agonists and antagonist. GABA concentrations used was 1, 5, 10, 20, 50, 100, and 500 μM, β-alanine concentrations used was 1, 5, 10, 20, 50, 100, and 500 mM, and bicuculline concentrations of 0.01, 0.1, 1, 5, 10, and 100 μM was co-applied with 100 μM GABA.

System operation. The fabrication of the devices has been described in detail elsewhere (16). The DF-48 chip is comprised of 48 sample wells connected to an open volume through 50x60 μm (wxh) microchannels. At the exit into the open volume, the channels are tightly packed, separated by 30 μm wide walls. The chip was placed on a software controlled, motorized scanning stage mounted on an inverted microscope. A cell was patch-clamped in the open volume, lifted up approximately 30 μm, and translocated to the channel outlets. Pressure was applied to initiate a flow corresponding to 3 mm/s, and the cell was translocated between different solution environments and exposed to ion channel effectors according to a predefined experimental protocol.

Results and discussion

We present a microfluidic device designed to increase throughput in ion channel screening. The system is composed of a 48-well microfluidic chip, a motorized scanning stage, a pump for creating flow, and software to control the scanning actions of the motorized scanning stage. The system is easily integrated onto the microscope of an existing patch clamp setup (Figure 1). Due to the small size of the microchannels, the fluids behave in ways that differ in a fundamental way from macroscopic systems. The flow in the microfluidic device is in the low Reynolds number regime, characterized by a completely laminar flow, with mixing occurring only by diffusion (17). As a result of this, the individual channel compositions is maintained well out into the open volume (16). Thus, virtual microcontainers of different drug solutions are created in an open volume that can be accessed by a biological cell detector only a few microns large, e.g. a patch clamped cell. By translating a lifted, patch clamped cell in front of the channel outlets, solution switch times of 10-15 ms is well achievable. Apart from the advantages in ion channel screening, several other benefits are achieved by placing the patch clamped cell in the laminar flow. The force that the laminar fluid flow exerts on the cell-pipette system stabilizes the seal and produces higher seal resistances (without affecting the access resistances), longer recording times, and lower noise levels (18).

The system was used together with WSS-1 cells expressing the $GABA_A$-receptor, chloride selective ion channels that become activated after binding a wide variety of agonists (19). Investigation of the receptor function was performed using a device containing 48 separate channels enabling the sequential exposure of a single cell to 48 different solutions, allowing the determination of several distinct dose response curves from the same cell using different agonists and antagonists with varying properties for the activation

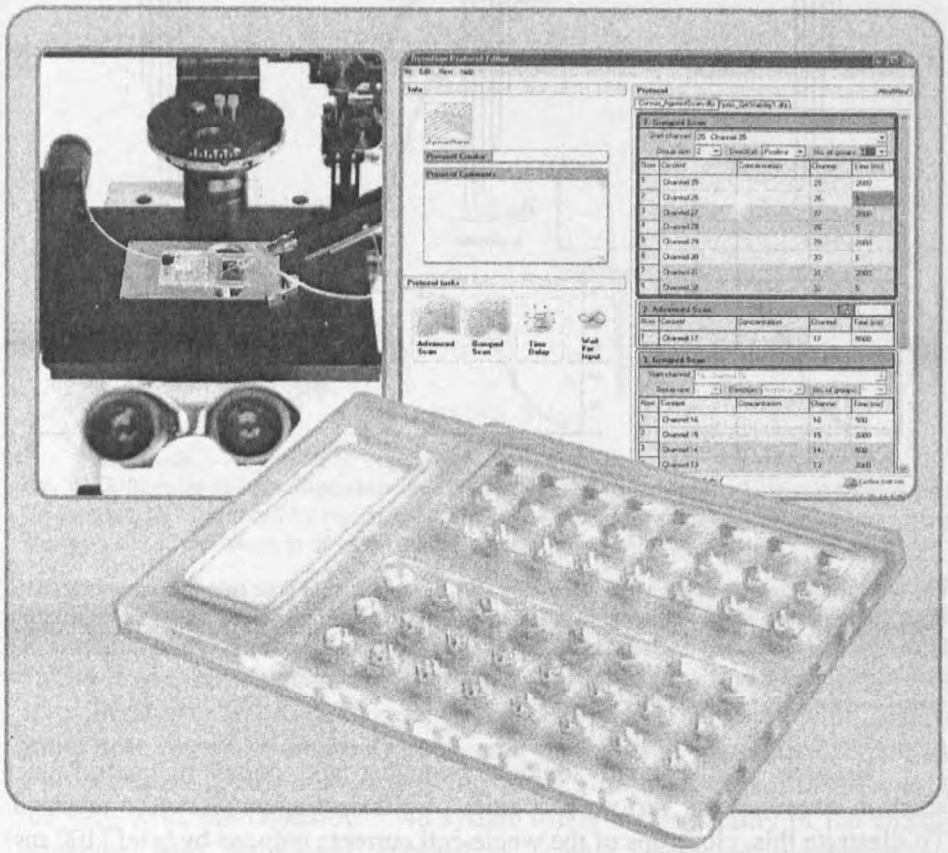

Fig. 1. Components of the DF48 system. The microfluidic chip (*bottom*) is controlled by a versatile software (*top right*) and integrates easily into an existing microscope set-up (*top left*).

kinetics of the receptor (Figure 2). The ion channels were activated by sequential exposure to seven different concentrations of GABA and β-alanine, respectively, followed by six different concentrations of the antagonist bicuculline, co-applied with 100 μM GABA. Due to the greater number of channels available it is possible to record complete dose response curves, for several different agonists, from the same cell. In this way, cell to cell variation is eliminated, producing better data quality along with the possibility to compare the effects of different agonists and antagonist more clearly, since the same cell is used throughout the recording. At the same time, the applied flow results in longer recording times and improved recording parameters.

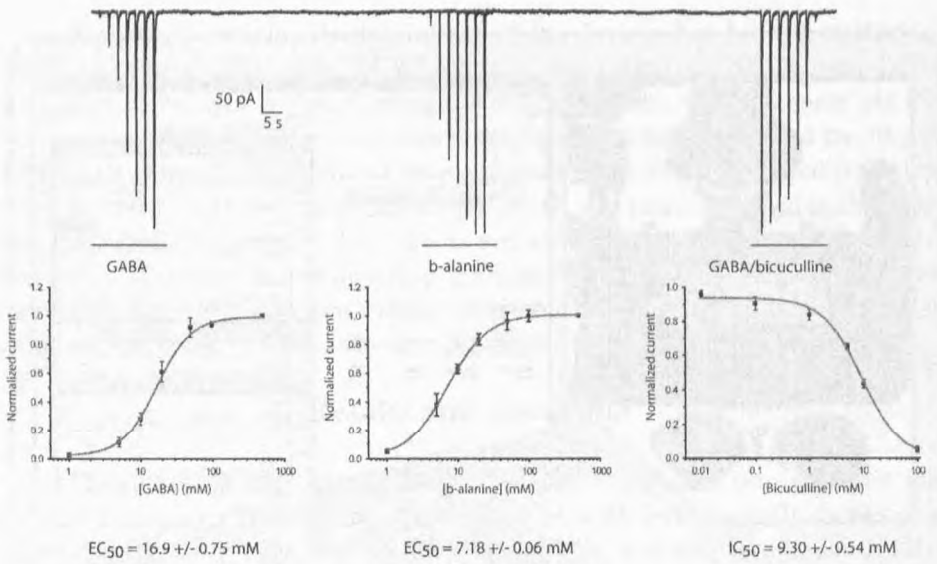

Fig. 2. Multiple agonist application to the same cell. Three different dose response curves recorded from the same cell in one continuous trace. A lifted patch clamped WSS-1 cell expressing the $GABA_A$ receptor was exposed to three different sets of antagonist and/or agonist and the resulting current was recorded. Lower part shows dose response curves obtained by plotting peak current versus concentration of agonist or antagonist (n=3).

Because of the rapid solution switching and complete control over solution environments various properties of different agonists can be resolved. To illustrate this, close-ups of the whole-cell currents induced by brief (100 ms) applications of saturating concentrations of GABA and β-alanine respectively are shown (Figure 3). The onset of current is the same for both agonists whereas the more rapid unbinding of β-alanine (20,21) is clearly seen during the return to baseline phase, where the GABA induced current returns much slower as compared to the β-alanine induced current. Thus, using the system described here to probe ion channel function, it is possible to resolve differences in unbinding rates of different ligands. The system has also previously been used successfully for extremely rapid ion channel systems such as AMPA/kainate, NMDA, and nicotinergic α7 ion channels, demonstrating the versatility of the system with regards to experimental systems requiring high temporal resolution.

In conclusion, the Dynaflow™ 48 microfluidic system makes it possible to record a large number of dose response curves quickly, with the added benefit of producing longer recording times and lower noise levels. The described microfluidic chip system offers robust and reliable detection with major

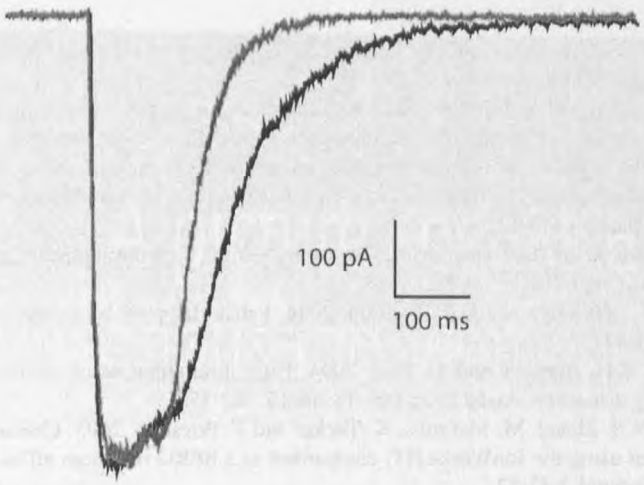

Fig. 3. Differences in unbinding rates are clearly resolved. Close up of whole cell kinetics from $GABA_A$ receptors, induced by rapid application of GABA (black trace) or β-alanine (grey trace). The pronounced difference in off-rate between the two agonists is clearly seen.

increases in throughput for conventional, high-quality patch-clamp screening of ion channel functions. Moreover, the 48 channel layout allows for several different agonists, antagonists or combinations thereof to be applied to the same cell, eliminating cell to cell variation. Data quality is improved by the fact that entire dose responses are easily obtained from the same patch-clamped cell and with increased signal-to-noise ratio due to the flow stabilization of the seal. In the long term, the Dynaflow™ 48 system offers the possibility of ion channel screening with maintained information content, increased throughput and decreased cost.

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