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A method for bidirectional solution exchange—"Liquid bullet" applications of acetylcholine to α7 nicotinic receptors☆

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ABSTRACT

Fast solution exchange techniques have revolutionized the study of synaptic transmission and promise to remain an important neuroscience research tool. Here we provide evidence for the hypothesis that using continuous, rapid transitions through an agonist solution can significantly increase the exchange rate around a cell by reducing the diffusion boundary at the membrane. This novel approach of rapid solution exchange during whole-cell recordings – described as a "liquid bullet" (LB) application – takes advantage of a bidirectional solution flow around the cell, allowing for a full solution exchange within a range of several milliseconds. An exchange rate (10–90% rise time) of about 2 ms could be achieved during both agonist application and washout. We recorded whole-cell currents from cells expressing the rapidly desensitizing α7 neuronal nicotinic receptor (NNR) subtype that exhibited very fast rise times of around 4–5 ms. We further demonstrated the advantages of a LB application over conventional methods by the ability of this method to elicit concentration-dependent responses for rapidly desensitizing compounds that were not measurable with conventional agonist applications. In addition, we illustrate the utility of this approach for frequency-based assays through fast, repeated agonist applications at frequencies of 1 Hz and 30 Hz. This approach could therefore be useful for the study of rapid agonist–receptor interactions that closely mimic the physiological conditions in the synaptic cleft during bursts of neuronal activity.

1. Introduction

Fast synaptic transmission can only be fully understood once the kinetics of neurotransmitter release and clearance are known, as well as the kinetic properties arising from the interactions between these transmitters and the corresponding receptors. The voltage-clamp technique has been used extensively to study the kinetics of voltage-gated channel activation and inactivation (e.g. Na^+ and K^+ channels), but until recently there was no equivalent technique for studying the kinetic properties of interactions between specific ligands and ion channel receptors. This situation was partly remedied with the development of the fast solution exchange technique (sometimes referred to as concentration-clamp), which has since revolutionized the study of synaptic transmission (Franke et al., 1987; Colquhoun et al., 1992).

The fastest known solution exchange rate achieved is around 100 μs with θ-electrodes mounted on a piezoelectric translator using excised membrane patches. These methods provide a significantly faster solution exchange rate (Franke et al., 1987) as compared to classical oocytes (Papke and Thinschmidt, 1998; Papke and Porter Papke, 2002) and cell based techniques (Sidach et al., 2009).

Several potential disadvantages however must be considered when applying the techniques of fast solution exchange to whole-cell configurations of recording. For example, unlike membrane patches, when a cell is moved between two fluidic environments, the situation becomes more complex due to the dragging along of a greater volume of fluid (Landau and Lifshitz, 1987). Second, there is a substantial distortion of the solution interface during whole cell crossing. Third, a rapid flow of solution could potentially result in a turbulent area on the side of the cell opposite to the solution exposure, leading to slower exchange rates. These three potential drawbacks could have a substantial impact on the activity of fast-desensitizing receptors such as α7 nicotinic receptors, which have been shown to be significantly desensitized during the peak amplitude of currents recorded in oocytes (Papke et al., 2000), as well as in faster, cell-based solution exchange

Abbreviations: NNR, neuronal nicotinic receptors; LB, liquid bullet; ACh, acetylcholine.

☆ Dynaflow is a registered trademark of Cellectricon AB.

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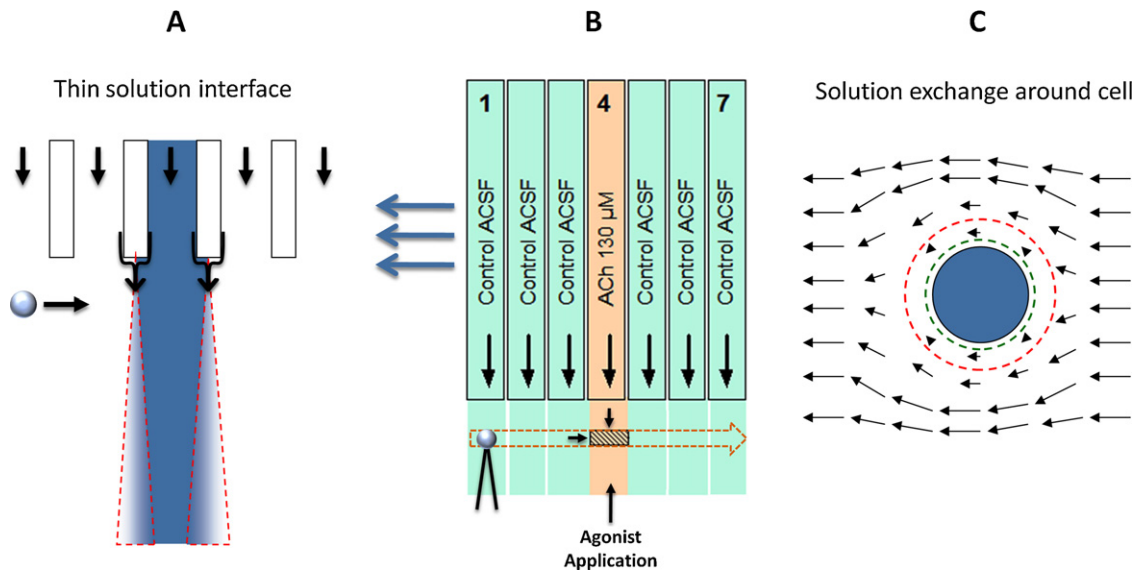

Fig. 1. Depiction of liquid bullet application and protocol. (A) The microfluidic chip creates individual solution flows with very narrow diffusion zones between each channel (framed by red dashed lines). The cell is scanned perpendicular to the flow from the channels. Typically the diffusion zone to be passed is 10–20 μm where the cell is scanned. (B) An illustration of the Dynaflow® chip settings used to produce a "liquid bullet" bi-directional application of agonist. Seven channels are pressurized to produce a continuous flow (black arrows) of both control (green) and agonist (pale orange) solutions. The dashed orange arrow shows the relative course of exposure of the cell across the channels as the chip is moved (direction of the chip movement is illustrated by the blue arrows). The cross-filled area represents the section of agonist-containing solution that is crossed by cell and termed a "liquid bullet" application due to its discrete exposure boundaries. This "liquid bullet" application results in a bi-directional solution exchange around the cell during agonist exposure, shown in (C). Solution exchange around a cell is highly affected by the total flow around it. Closest to the cell, the flow velocity is always zero due to viscous forces. This means that there will always be a boundary radius outside the cell, inside which mass transport is dominated by diffusion. For slow flow (red dashed line), this boundary layer is far from the cell surface, and thus diffusion transport will take longer due to the long distance to be passed. For fast flow (green dashed line), the distance is short, therefore the diffusion time is small.

systems, although to a somewhat smaller extent (Grady et al., 2010).

Traditional ligand-application setups have in fact not shown a fast enough solution exchange to properly resolve rapidly desensitizing receptors in a whole-cell recording configuration. It is apparent that both a boundary layer created by the fluid being dragged along the surface of the cell (Landau and Lifshitz, 1987) and poorly defined solution interfaces can substantially reduce the rate of solution exchange on a millisecond time scale. In this case, a bidirectional laminar solution flow using laminar flow from closely distanced channels to create narrow diffusion zones between the defined solutions could be beneficial (Fig. 1A). To explore the potential advantages of this technique, we first designed experiments where we created a continuous and uninterruptable bidirectional solution flow around a cell during and after the application of agonist using a technique we describe as a "liquid bullet" (LB) application (Fig. 1B). Second, we measured the rate of α7 receptor activation under these experimental conditions and compared it with data from literature. This method was used to demonstrate the utility and several potential advantages of a fast, bidirectional solution exchange for studying rapidly desensitizing α7 NNRs in response to their endogenous ligand acetylcholine (ACh), as well as to another rapidly desensitizing compound (Bencherif and Lippiello, 2010).

2. Materials and methods

Detailed methods and solution composition have been described previously (Sidach et al., 2009; Fedorov et al., 2009). Briefly, after removal from the incubator, cells were washed twice with extracellular recording medium (NaCl, 130 mM; KCl, 5 mM; $CaCl_2$, 2 mM; $MgCl_2$, 2 mM; glucose, 25 mM; HEPES, 10 mM, pH 7.4) and placed into a 48 channel Dynaflow® chip (Cellectricon, Inc.). Chips were placed on the Dynaflow® stage of an inverted Zeiss microscope at room temperature. Borosilicate electrodes were filled with: Tris–phosphate dibasic, 110 mM; Tris–base, 28 mM; EGTA, 11 mM; $MgCl_2$, 2 mM; $CaCl_2$, 0.5 mM; NaATP 4 mM, pH 7.25 and had resistance of 2–5 MΩ. Cells were held at −60 mV and α7 currents were recorded with an Axopatch 700A amplifier, filtered at 1 kHz, and sampled at 5 kHz (Digidata1440, Molecular Device, Inc.). On average, the whole-cell recording stabilized within <5 min. "Conventional" responses were evoked by moving the cell in front of the agonist-containing channel for 1 s, and 30 s washout periods were used between applications (Grady et al., 2010).

2.1. Cell culture

A CHO cell line with stably expressed human α7/RIC3 receptors was purchased from Chantest Inc. (catalog # CT6201). Cell handling procedures were carried out according to the provider's recommendations. Briefly, cells were seeded at $\sim 9 \times 10^4$ density per 100 mm plate and 70% confluence was reached in 3–4 days. The media contained fetal bovine serum (10%), penicillin–streptomycin (100 units/mL), geneticin (G418, 0.25 mg/ml) and Zeocin (0.4 mg/mL, Invivogen). 2.5 ml of 0.25% trypsin/EDTA (Mediatech, Inc.) was used to lift cells off the bottom of the plate (3–4 min) at room temperature, followed by gentle titration of the cells.

2.2. Design of bidirectional solution flow

To achieve a bidirectional solution exchange, a continuously high-velocity flow around the cell needed to be introduced. One potential way to achieve this was to use the relative movement of a cell at a constant speed in a perpendicular direction across a steady laminar flow of solution. To attempt this, we used an existing, commercially available fast solution exchange system, Dynaflow® (Fig. 1B). To distinguish this type of application from a conventional solution application, we will refer to this technique as a liquid bullet (LB) application (Fig. 1B).