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A Chip-Based Stable Lipid Bilayers for Recording hERG Channel Activities

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Abstract

The human *ether-a-go-go*-related gene (hERG) channel, a cardiac voltage-dependent potassium channel, has been drawing a lot of pharmacological attention because it has been found to cause serious arrhythmic side effects. Here we report on the reconstitution of hERG channels in artificial bilayer lipid membranes (BLMs) formed in micropores fabricated in silicon (Si) chips. The hERG channels were isolated from Chinese hamster ovary (CHO) cell lines expressing the channels and incorporated into the BLMs formed by a process in which the two lipid monolayers were folded into the micropores. The characteristic features of hERG channels reported by the patch-clamp method, including single-channel conductance, voltage dependence and sensitivity to typical drugs, were observed in the BLM reconstitution system. The BLM with hERG channels incorporated exhibited a lifetime of ~65 h and a tolerance to repetitive solution exchanges. Such stable BLMs containing biological channels have the potential for use in a variety of applications, including high-throughput drug screening for various ion-channel proteins.

1. Introduction

Ion-channel proteins are of crucial physiological importance and are major targets for drug design. Recording ion-channel activities by measuring ion currents after reconstitution of ion-channels in artificial bilayer lipid membranes (BLMs) represents a simple method to investigate channel function and to screen the effect of drug candidates. Recently, there is growing interest in the development of high-throughput safety screens targeted for ion channels, since the hERG potassium channel is often adversely affected by drugs that are designed for totally unrelated targets, causing a potentially fatal arrhythmia. Regulatory agencies now require that the potential for drug candidates to inhibit cardiac ion-channels be assessed, particularly hERG, before clinical trials are performed. BLM in which the hERG channel is incorporated, represents a potentially new technique for screening substances that directly block hERG channels. In the present study, we incorporate hERG channels into stable BLMs formed in micropores fabricated in Si chips [1]. Ion currents of single or multiple hERG channels were investigated in terms of single-channel conductance, voltage dependence and sensitivity to typical drugs.

2. Experimental Section

CHO cell lines expressing hERG channels (Kv11.1) were obtained from the Channelopathy Foundation (Basel, Switzerland). The hERG channels were extracted from CHO cell lines as membrane fractions. Micropores with a diameter of 20-60 μm were fabricated in Si chips [2] and the surface of the chip was coated with Teflon-AF and thermal oxide [3]. BLMs were prepared by the monolayer folding method across the Si chips (Fig. 1). The composition of the lipid solution was 2 mg/ml L-α-phosphatidylcholine: L-α-phosphatidylethanolamine: cholesterol = 7:1:2 (w/w) in chloroform/*n*-hexane (1:1, v/v). The incorporation of hERG channels into the BLMs was performed by fusing hERG-containing proteoliposomes either with the preformed BLMs or with the lipid monolayer on the solution before BLM formation. Buffer solution used for recording channel activities of hERG was 120 mM KCl and 10 mM HEPES/KOH (pH 7.2). Current recordings were performed with an Axopatch 200B patch-clamp amplifier (Molecular Devices). Signals were on-line filtered at 1 kHz with a low-pass Bessel filter, digitized at 10 kHz, and stored on-line using a data acquisition system (Digidata 1440 and pCLAMP 10.2, Molecular Devices). The currents were off-line filtered at a cut-off frequency of 0.7 kHz.

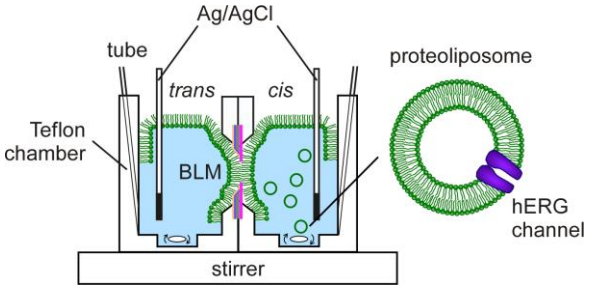

Fig. 1 Schematic of a BLM formed across a micropore in the present Si chip.

3. Results and Discussion

The hERG channel is a voltage-gated potassium channel that is crucial for repolarization during action potentials in human ventricular myocytes. We first examined record-

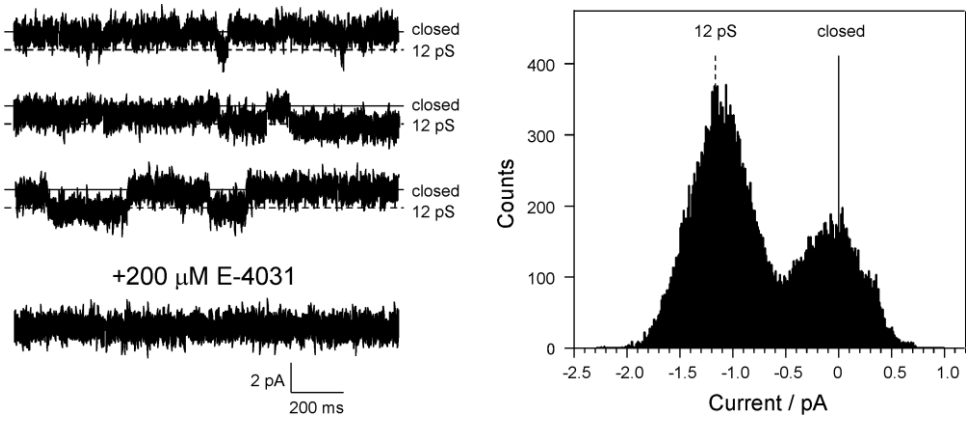

Fig. 2 Single hERG channel currents recorded at an applied potential of -100 mV.

ings of the hERG single-channel currents after incorporation of the hERG channel into BLMs formed in the microfabricated Si chip (Fig. 2). Stepwise currents were evident, with an average single-channel chord conductance of 11±1.2 pS (n=3). This conductance level is similar to a previously reported value obtained using the patch-clamp method [4]. The addition of E-4031, a specific blocker, completely blocked channel activity, confirming the functionality of the hERG channel incorporated in the preset BLMs. Single-channel activity was only observed at negative applied potentials. At positive potentials, no outward currents were observed, which is in agreement with a previous report by the patch-clamp method [4]. Artificial BLMs formed in microfabticated Si chips have been criticized from the standpoint that the BLMs exhibit a high background noise level due to the large device capacitance. However, the use of a SiO_2/Teflon dielectric layer greatly reduces the noise level, which permitted single-channel activities of the hERG channel, whose conductance is relatively low, to be recorded.

Fig. 3 shows the effects of E-4031 and astemizole on hERG currents at the single-channel level. Astemizole is an antihistamine that has been withdrawn from the market in most countries due to its side effect on the hERG channel. Before the addition of the drugs (control), stepwise currents were observed with a single-channel conductance of ~13 pS. The addition of E-4031 completely blocked the current, thus confirming that the control currents stemmed from the permeation of ions through the hERG channel. After a thorough washout of E-4031, the channel activities were recovered. Finally, the addition of astemizole again completely blocked the channel current, confirming its inhibitory effect on hERG channels reconstituted in a BLM. Thus, the sensitivity of the hERG channel to most typical drugs has been successfully reproduced in the present BLM reconstitution system. In addition, the channel activities were observed after ~65 h, demonstrating that present BLM with integrated ion channels is stable and retains high-sealing properties for long hours.

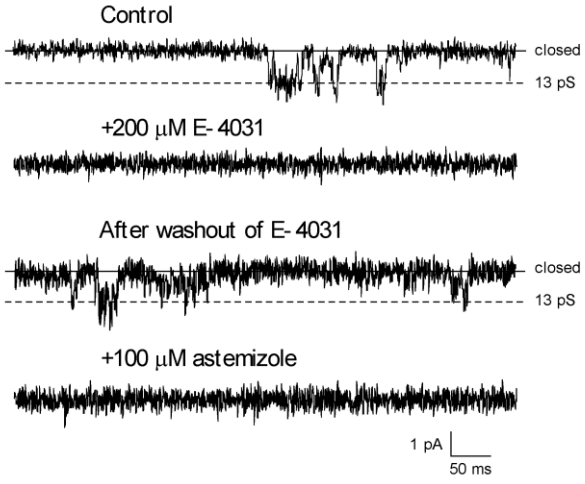

Fig. 5 Blocking of a single hERG channel by E-4031 and astemizole.

3. Conclusions

We report herein on the intact incorporation of hERG channels into artificial BLMs suspended in microfabricated Si chips. The characteristic features of hERG channels, such as single-channel conductance, voltage dependence and sensitivity to typical drugs were retained after being incorporated into the BLM in Si chips. These results demonstrate the significant potential of the present hERG-based Si chip as a platform for drug safety screens.

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