Compound adsorption and the Prevention Measure in the Automated Patch-Clamp Assay

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1. Introduction

Automated patch-clamp systems have dramatically improved efficiency of the hERG assay to detect drugs' potency of QT-interval prolongation that may lead to induction of life-threatening ventricular arrhythmia, torsade de pointes, and cardiac sudden death. However, it has become clear that the experimental results of some of known hERG-channel blockers in the automated patch-clamp system differ from those obtained in the manual patch-clamping. Concentration-response curves of the hERG-channel blockers obtained in the automated patch-clamp system shifted in the higher-concentration direction compared to those obtained in the manual patch-clamping. This fact may affect the reliability of the hERG assay using the automated patch-clamp system, because the potency of a compound in inhibiting hERG currents could be underestimated.

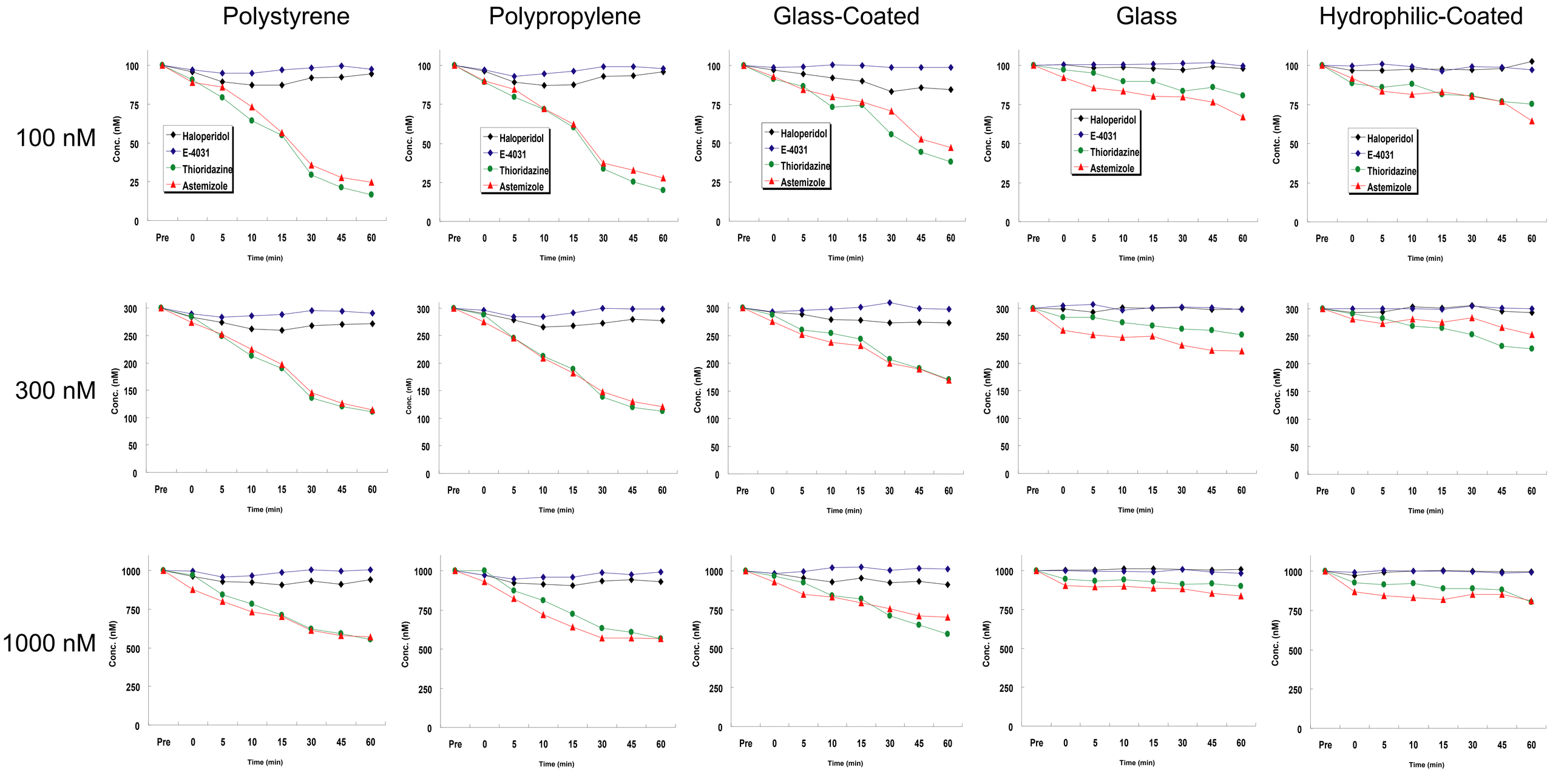
In this study, we investigated the possibility of 4 known hERG-channel blockers to adsorb into 96-well plates that are used to store test-substance solutions in the automated patch-clamp system. Five types of 96-well plates made of various materials were compared in terms of the time course of concentration of hERG-channel blockers in the test-substance solutions placed in the respective plates. Subsequently, we confirmed the influence of adsorption of a hERG-channel blocker into the 96-well plate on the concentration-response of compounds for the hERG-current inhibition.

2. Materials and Methods

The time course of concentrations of 4 known hERG-channel blockers, haloperidol, E-4031, thioridazine and astemizole, in test solutions were determined by HPLC assay. Then, the results were compared between 5 types of plates, polystyrene, polypropylene, glass, glass-coated plastic, and hydrophilic-coated plastic. Test solutions were prepared as follows: First, the test substance was dissolved in dimethyl sulfoxide to yield concentrations of 100, 300, and 1000 μM. These solutions were then diluted 1000 times with the extracellular superfusing solution. A total of 300 μL of the test solutions at the respective concentrations were placed in wells of the 96-well plates; then, 200 μL each of the test solutions was sampled with time. A total of 8 sampling time points, ie, before placing in wells, 0, 5, 10, 15, 30, 45, and 60 minutes after placing, were set. Three samples were taken at the respective time points and these sampled test solutions were thoroughly mixed with 200 μL of methanol, and their concentrations were measured using HPLC (Hitachi High-Technologies Corporation).

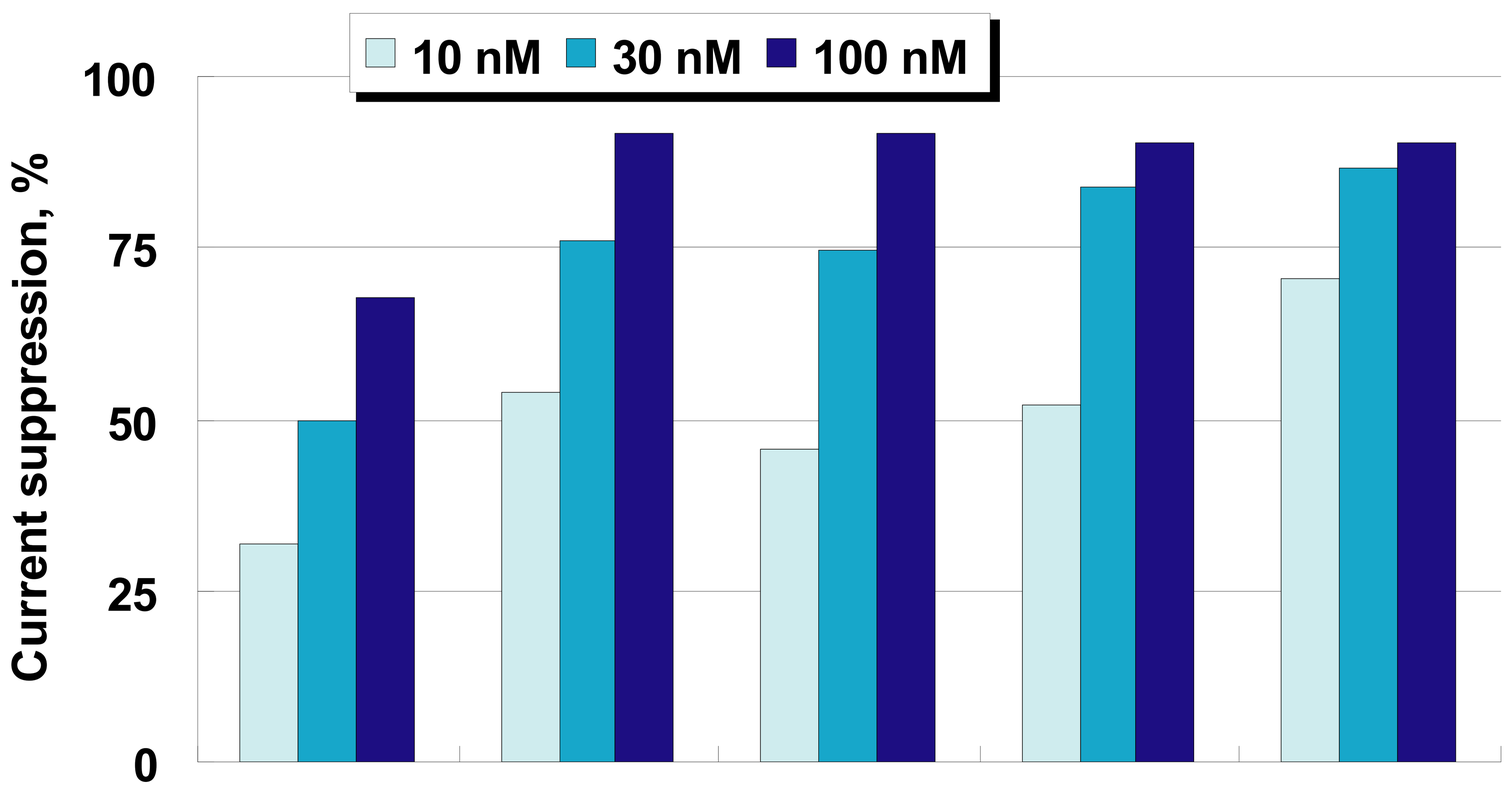
The influences of the adsorption of astemizole on the concentration-response for the hERG-current inhibition were investigated by the whole-cell patch-clamp technique using the automated patch-clamp system, PatchExpress 7000A (Molecular Devices Corporation). Differences in the hERG-current-inhibition level of astemizole at 10, 30, and 100 nM with the aforementioned 5 types of 96-well plates were assayed.

3. Time Course of Concentrations of hERG-Channel Blocker in 96-well Plates



The time course of concentrations of 4 known hERG-channel blockers in test solutions stored in 5 types of plates were determined by HPLC assay. The concentrations of a test-substance in the test solution were plotted as a function of durations to keep the solution in the 96-well plate.

4. Effects of Astemizole on the hERG Potassium Channels



Concentration-response relationships of astemizole on hERG potassium channel currents when test solutions were stored in 5 different types of 96-well plates for 60 min prior to application. The test solution was injected into an application chamber cumulatively from lower concentration and the test solution in the application chamber was maintained at room temperature. The value of current used to determine the effects of the compound was the peak-outward-tail-current amplitude measured at -50 mV repolarization for 5 s following the initial depolarizing step to 20 mV for 5 s from a holding potential of -80 mV. These pulses were given every 12 seconds. Mean percentage of current suppression in 2 cells is shown in the bar graph.

5. Conclusion

The concentrations of thioridazine and astemizole in the 96-well plate made of polystyrene and polypropylene remarkably decreased in a time-dependent manner, whereas the those of haloperidol and E-4031 did not change so much. The concentration decreased more at lower concentrations than at the highest concentration of 1000 nM and when stored longer, thus the adsorption of thioridazine and astemizole into the 96-well plate was concentration- as well as time-dependent. The concentrations of thioridazine and astemizole increased when test solutions were stored in the plates made of glass, glass-coated plastic, and hydrophilic-coated plastic. The subsequent hERG assay using the automated patch-clamp system showed higher current suppression with these two plates compared to others. Among the 96-well plates tested in the preset study, the plates made of glass or hydrophilic-coated plastic seems to most suitable to store test solutions in the automated patch-clamp system.