HTS Screening of hERG Blockers Using 2nd-Generation-Automated Patch-Clamp System

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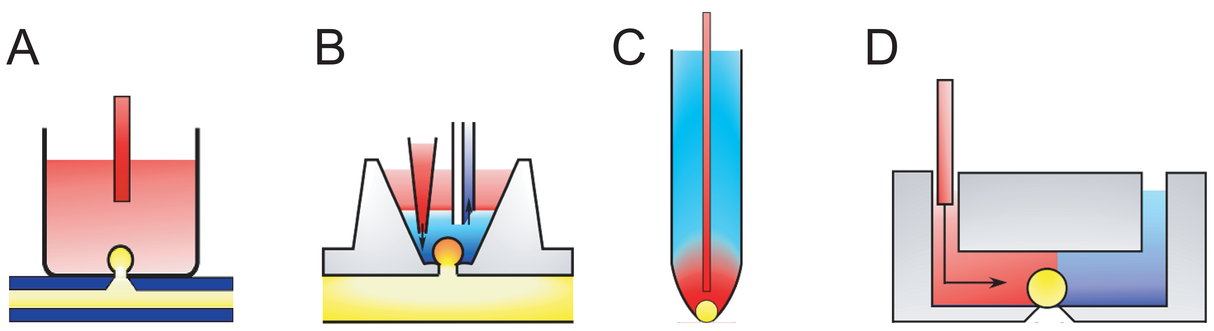
Introduction

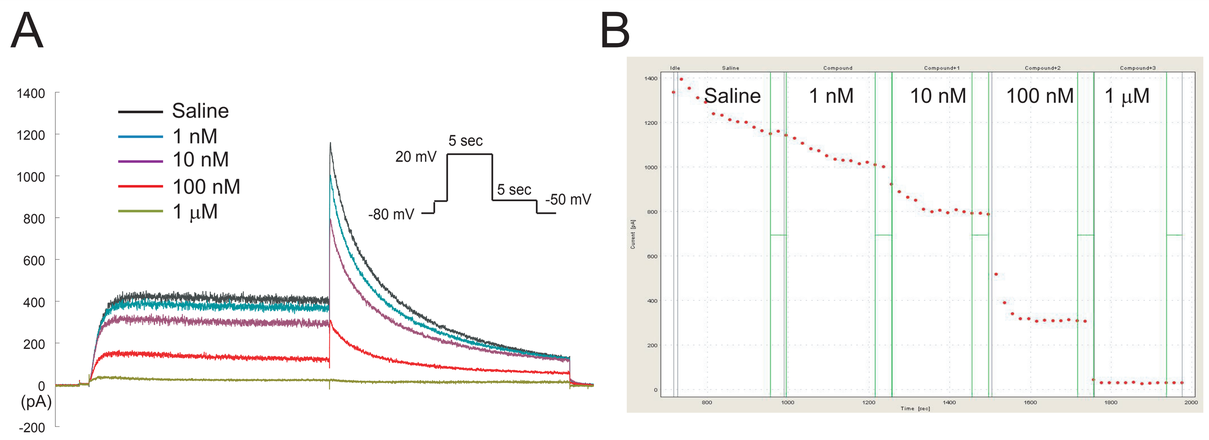
As a part of safety pharmacology studies, screening of drug candidates to seek their potency in blocking the hERG channel has become more important and the need of high throughput technology for the electrophysiological testing, such as patch-clamp experiments, has been stimulated. The automated patch-clamp screening system has been being designed and developed by many scientific instruments manufacturers in order to dramatically enhance efficiency of the patch-clamp experiments and some of the systems are now in operation in the pharmaceuticals industries. However, in some hERG-positive compounds, it had been unveiled that dose-response curves obtained in the automated patch-clamp system were shifted into the higher concentrations compared to those in the conventional patch clamping due to compounds' adsorption into experimental apparatuses. Recently, application technologies flowing test compound solutions within the application chamber have been developed for the automated patch-clamp system in order to reduce possibilities of compounds' adsorption into the application route and the automated patch-clamp system equipped with these new technologies have been launched as second generation of the automated patch-clamp system. In this study, the dose-dependent effects of drugs on the hERG channel transfected in CHO cells were determined using a second-generation-automated patch-clamp system, QPatch 16.

Application Technologies in Automated Patch-Clamp System



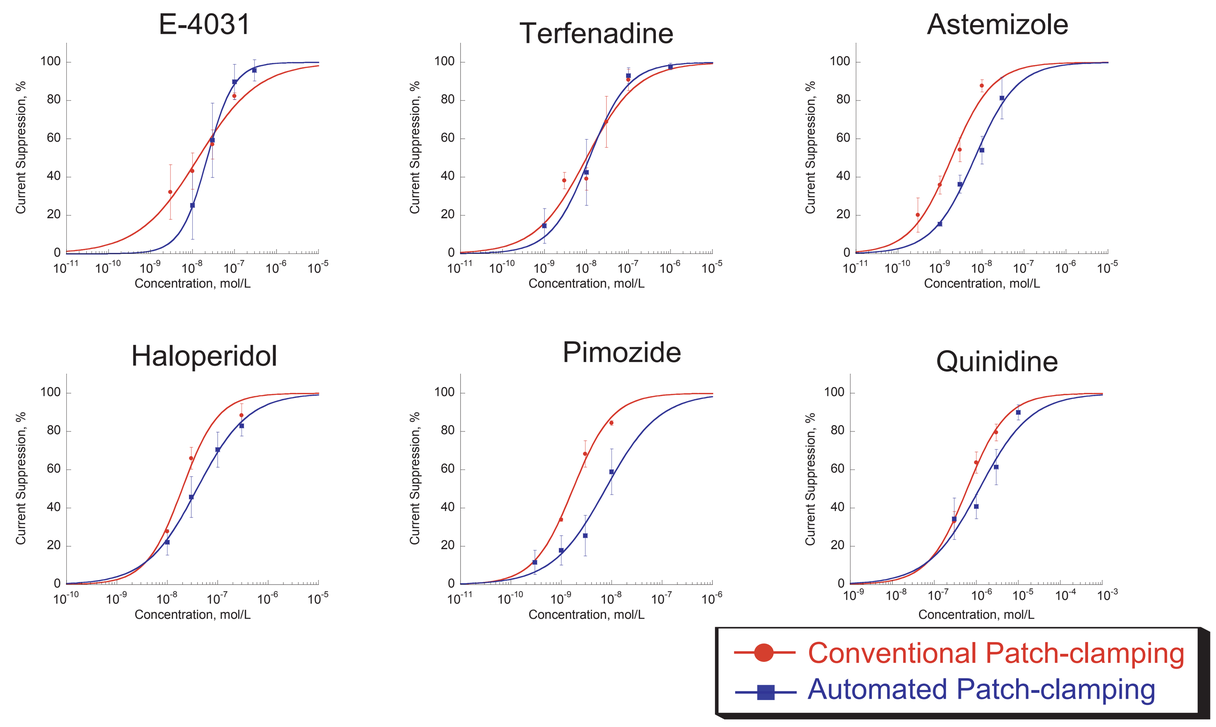
Schematic diagrams of the test substance application in the various automated patch-clamp systems. A; Concentrated test substance solution is infused into the application chamber and the test substance diffuses in the extracellular solution (e.g. IonWorks). B; Extracellular solution is removed by aspiration, and then test substance solution is injected into the application chamber (e.g. PatchXpress). C; Test substance solution is infused locally around a cell that forms the whole-cell configuration (e.g. Flyscreen). D; Test substance solution is flowed into narrow channel by laminar action to replace the extracellular solution (e.g. QPatch 16).

Effects of Terfenadine on the hERG Channel



Effects of terfenadine on the hERG channel in QPatch 16 system. A; The hERG current recorded before and after application of terfenadine in QPatch 16 system. The pulse command shown in the inset was injected to the cell every 15 seconds. Current records from a single CHO cell before (black line) and after application of terfenadine (colored lines) are superimposed. B; Time course of peak tail current suppression in the absence and presence of terfenadine. Terfenadine was cumulatively applied from lower to higher concentrations in QPatch 16 system. Terfenadine showed time- and concentration-dependent hERG-current suppression.

Concentration-Response Relationships of hERG-Channel Blockers on the hERG Channel



Concentration-response relationships of hERG-channel blockers on hERG potassium channel currents in hERG transfected CHO cells using QPatch 16 system. The peak-outward-tail-current amplitude repolarized at -50 mV following the initial depolarizing step to 20 mV from a holding potential of -80 mV was used for determining the effects of a hERG-channel blocker. These pulses were injected every 15 seconds. The percent suppression of the hERG current is plotted as a function of the concentration of the hERG-channel blocker. Data were fitted using the Hill equation. Each point indicates the mean±SD (n=3 or 6).

IC_{50} Values of hERG Channel Blockers in hERG transfected CHO Cells

Test Substance	IC_{50} Value, nmol/L	
	Conventional Patch	Automated Patch
Astemizole	1.9	6.7
E-4031	14	18
Haloperidol	20	38.8
Pimozide	1.7	7.6
Terfenadine	18	25
Quinidine	607	1181
Verapamil	N.A	327
Clofilium	N.A	22

Concentration-response data were fitted to the Hill equation, that is, % inhibition = $100 / \{1 + (IC_{50} / [CB])^h\}$, where [CB], IC_{50}, and h represent concentration of a hERG-channel blocker, median inhibition concentration and the Hill coefficient, respectively (n=3 or 6). The IC_{50} value for the suppressive effect was calculated using a curve fitting program in KaleidaGraph 3.6 (Synergy Software).

Conclusion

The correlation between data obtained with automated patch-clamp (QPatch 16) and manual patch-clamp was high. The problem with overestimation of IC_{50} values due to 'sticky compounds' in the QPatch system seemed to be insignificant due to minimal exposure of the compound to plastic surfaces in the application chamber. These results demonstrated that the second generation of automated patch-clamp system would contribute to improvements of reliability and throughput of the hERG assay using the automated patch-clamp system for the prediction of drugs' potential risk of QT-interval prolongation.