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

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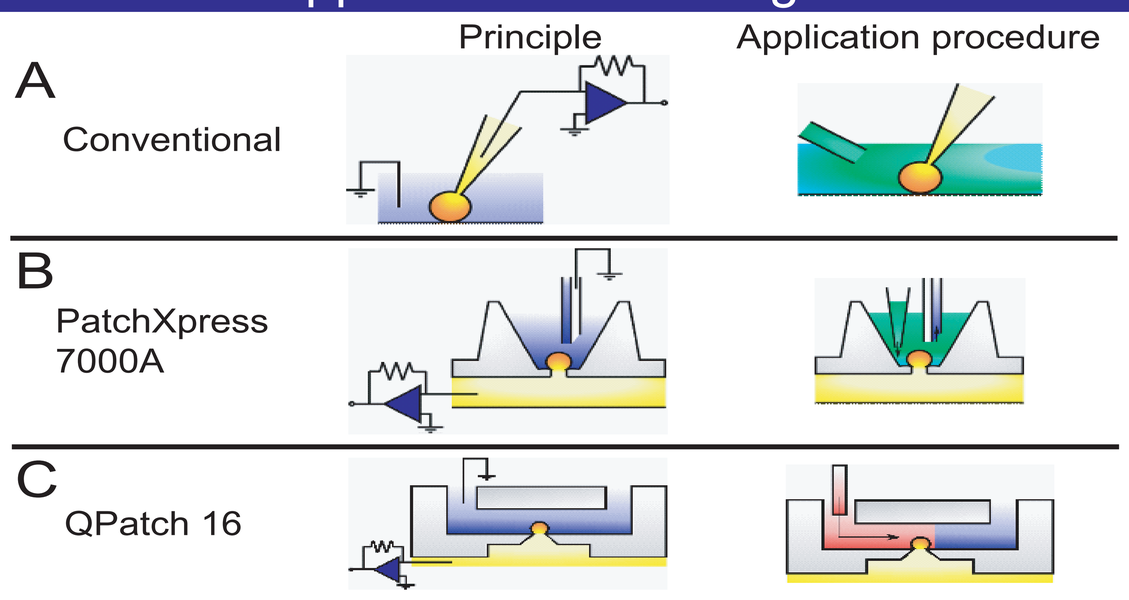
Introduction

The whole-cell patch-clamp assay using human ether-a-go-go-related gene (hERG) transfected cell lines has been well recognized as a part of safety pharmacology studies for evaluating effects of drugs to induce QT-interval prolongation. Since potassium currents passing thought the hERG channels can be measured directly by patch-clamping, the experiment is considered to be highly reliable to investigate the modulation of hERG channels by drugs. Many automated patch-clamp screening systems have been already developed and used in order to conduct the hERG assay as a high throughput screening. However, in some hERG-positive drugs, it had been unveiled that concentration-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 drugs' adsorption into experimental apparatuses. For the second generation of the automated patch-clamp system such as QPatch 16, an application technologies flowing test compound solutions within the application chamber have been developed to reduce possibilities of drugs' adsorption into the application route. Although, the correlation of the concentration-response relationship between QPatch 16 and the conventional patch-clamping was high in the most of positive-control drugs, thioridazine showed poor correlation. In this study, other possibilities than the adsorption of drugs to the application route to shift the concentration-response curve in thioridazine have been investigated.

Application Technologies



Schematic diagrams of the test substance application in the conventional and automated patch-clamp systems. A; Test substance solution can be continuously superfused into the application chamber. B; Extracellular solution is removed by aspiration, and then test substance solution is injected into the application chamber. C; Test substance solution is flowed into narrow channel by laminar action to replace the extracellular solution.

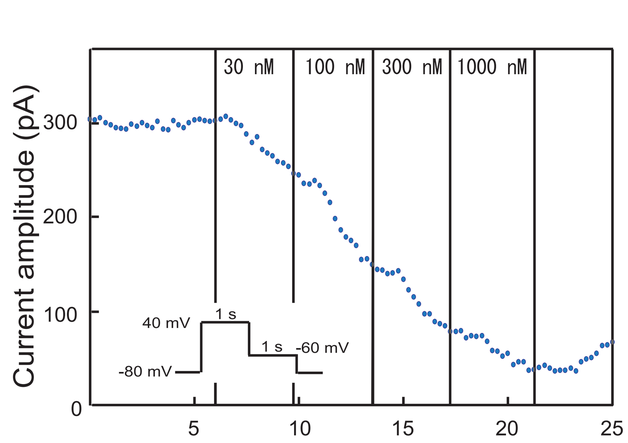
Effects of hRERG Blockers in Conventional Ptch-Clamp Systems

Test Substance	IC_{50} Value, nmol/L		
	HEK293 (WARF)	HEK293 (Cytomyx)	CHO (Cytomyx)
E-4031	20	14	14
Astemizole	3.4	2.1	1.9
Terfenadine	18	16	10
Pimozide	2.7	2.2	1.7
Haloperidol	25	34	20
Quinidine	579	571	607
Thioridazine	48	53	49

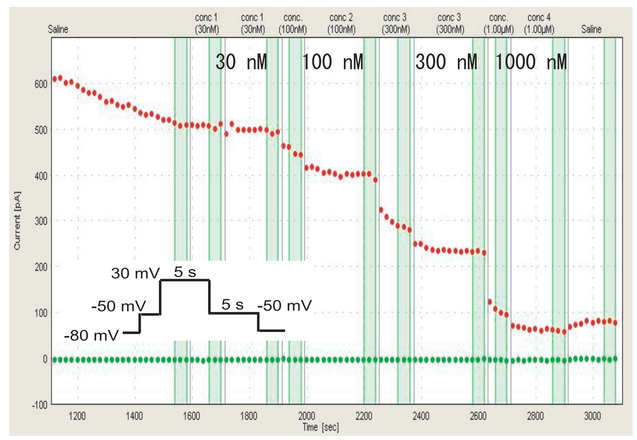
Concentration-response data were fitted to the Hill equation, % inhibition = $100/\{1 + (IC_{50}/[CB])^h\}$, where [CB], IC_{50} and h represent concentration of hERG-channel blocker, median inhibition concentration and the Hill coefficient, respectively (n=5 to 10). The IC_{50} value for the suppressive effect was calculated using a curve fitting program in KaleidaGraph 3.6 (Synergy Software).

Effect of Thioridazine on Cumulative Application

Conventional Patch-Clamp System

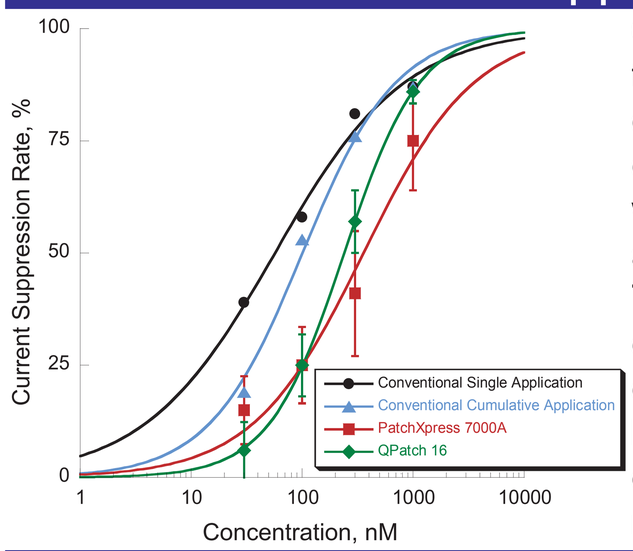


QPatch 16 System



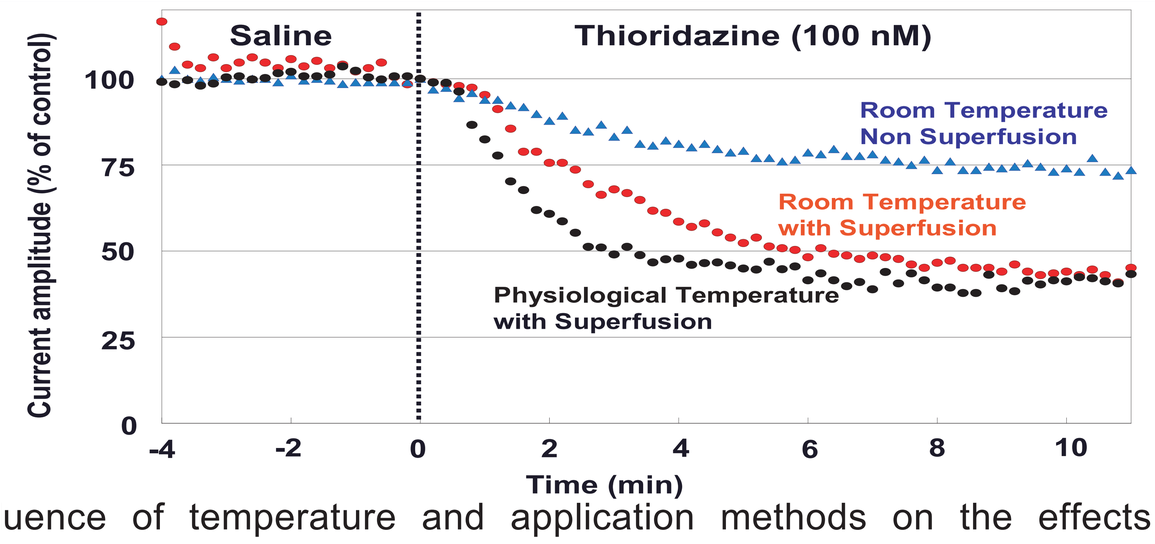
Time course of change of hERG potassium channel currents in hERG transfected CHO cells using the conventional patch-clamping and automated patch-clamp system when thioridazine was applied cumulatively. Voltage pulse protocols were run at intervals of 15 and 20 seconds in conventional patch-clamping and QPatch 16 system, respectively.

Concentration-Response Effects of Thioridazine in Different Application Methods



Concentration-response effects of thioridazine on hERG potassium channels in hERG transfected CHO cells in different application methods with the conventional patch-clamping and automated patch-clamp systems. 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=5 to 10).

Influence of Temperature and Application Methods on Effects of Thioridazine



Influence of temperature and application methods on the effects of thioridazine on the hERG potassium channels expressed in CHO cells with conventional patch-clamping. The superfusing solution containing thioridazine was superfused into an application chamber at a flow rate of 5 mL/min continuously or the superfusion was discontinued after achieving the concentration in the application chamber. The temperature of the superfusing solution was maintained at 37˚C or room temperature during the application.

Conclusion

The IC_{50} values obtained in the conventional patch-clamping and QPatch 16 system were well correlated in terms of the effects of hERG current suppression by most of hERG blockers besides thioridazine. In QPatch 16, the laminar flow of the test-substance solution with minimal exposure to plastic surfaces in the application chamber may contribute to the high correlation. Based on further study on thioridazine, it was reviled that the temperature and superfusion of extracellular solution are also factors to affect concentration-response relationship of the drug in the hERG current suppression. From these results, the functions for temperature control and extracellular superfusion are recommended for future upgrades of the automated patch-clamp systems.