High throughput hERG assay using the automated patch-clamp system; Optimization of experimental condition

Yoshimi Katayama, Kenji Tsuzaki, Keiko Yonezawa, Mariko Amagai, Kanae Abe and Yuji Tsurubuchi

Drug Safety Testing Center Co. Ltd., Saitama, Japan

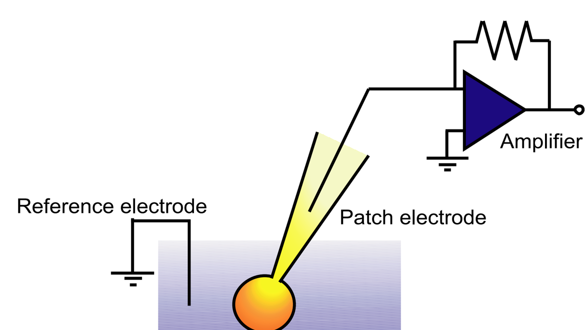
1. Introduction

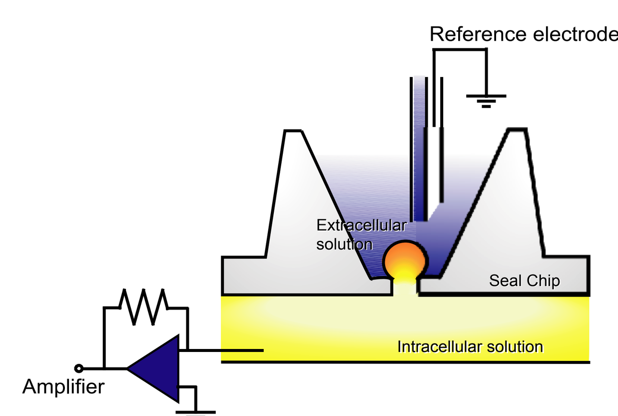
The whole-cell patch-clamp technique using human ether-a-go-go-related gene (hERG) transfected cell lines has become more important recently as a Safety Pharmacology Study to predict a potential risk of torsade de pointes in clinical use of drugs. Since potassium currents passing through the hERG channels can be measured directly by patch-clamping, the experiment is considered to be more reliable than other experiments such as the rubidium efflux assay. However, the patch-clamp experiment is very time consuming and the throughput is much lower than those of other assays. In order to dramatically enhance efficiency of the patch-clamp experiment, automated patch-clamp systems have been designed and developed by many scientific instrument manufacturers recently. In this study, an automated patch-clamp system, PatchXpress 7000A, was used to evaluate effects of drug on the hERG channels transfected in HEK 293 cells. Various voltage protocols to induce hERG currents were determined and the experimental conditions were optimized. Under the optimized conditions, the effects of standard drugs on the hERG channels could be assessed relevantly.

2. Methods

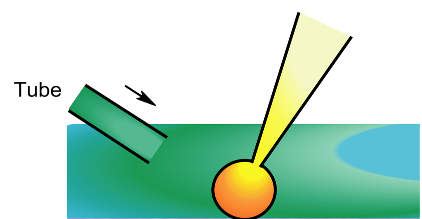
Conventional Patch-Clamp **PatchXpress 7000A**

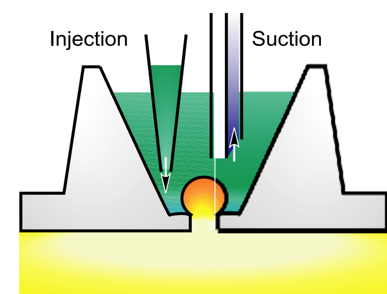
A. Principle of Measurement





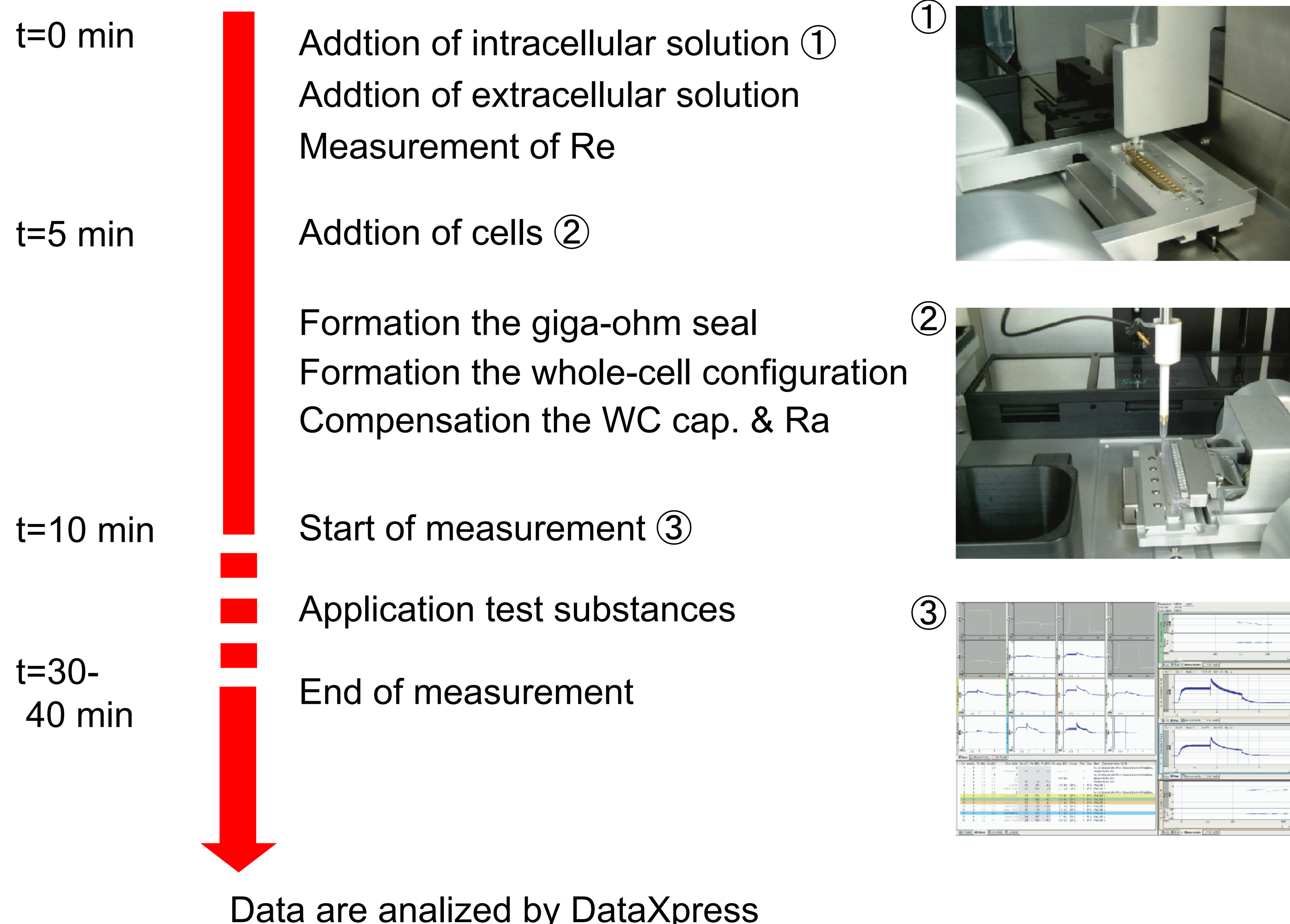
B. Exchange of Solution



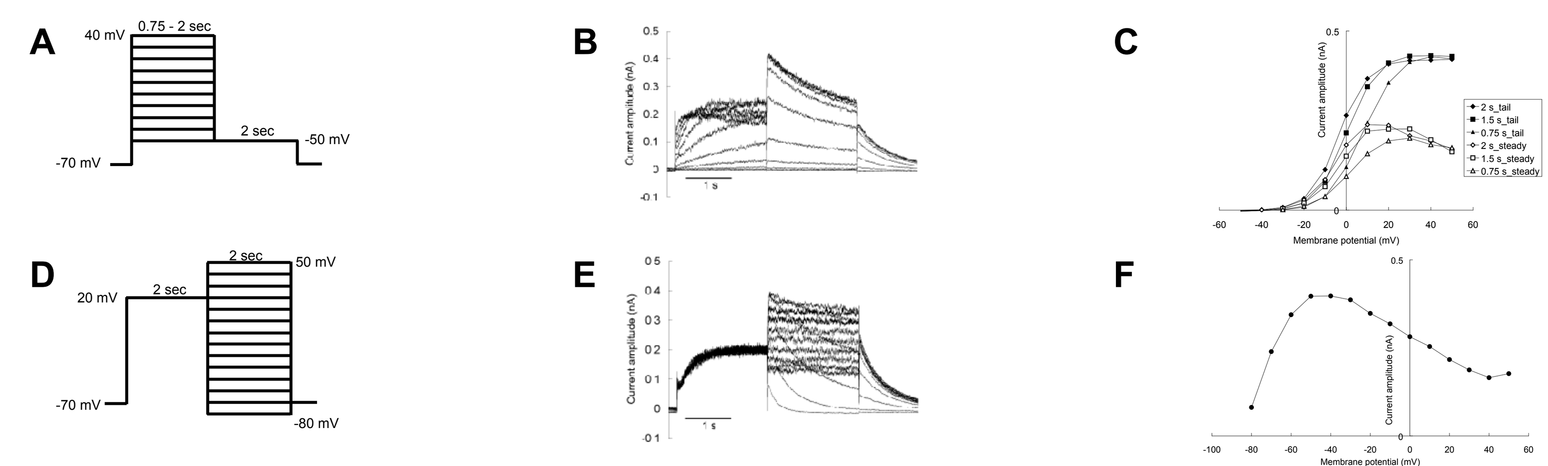


Methods used in the conventional patch-clamp technique and PatchXpress 7000A. A; Principle diagrams of measurement in the conventional and automated patch-clamping. B; Schematic diagrams of exchange of extra-cellar solution in the conventional and automated patch-clamping.

3. Procedure

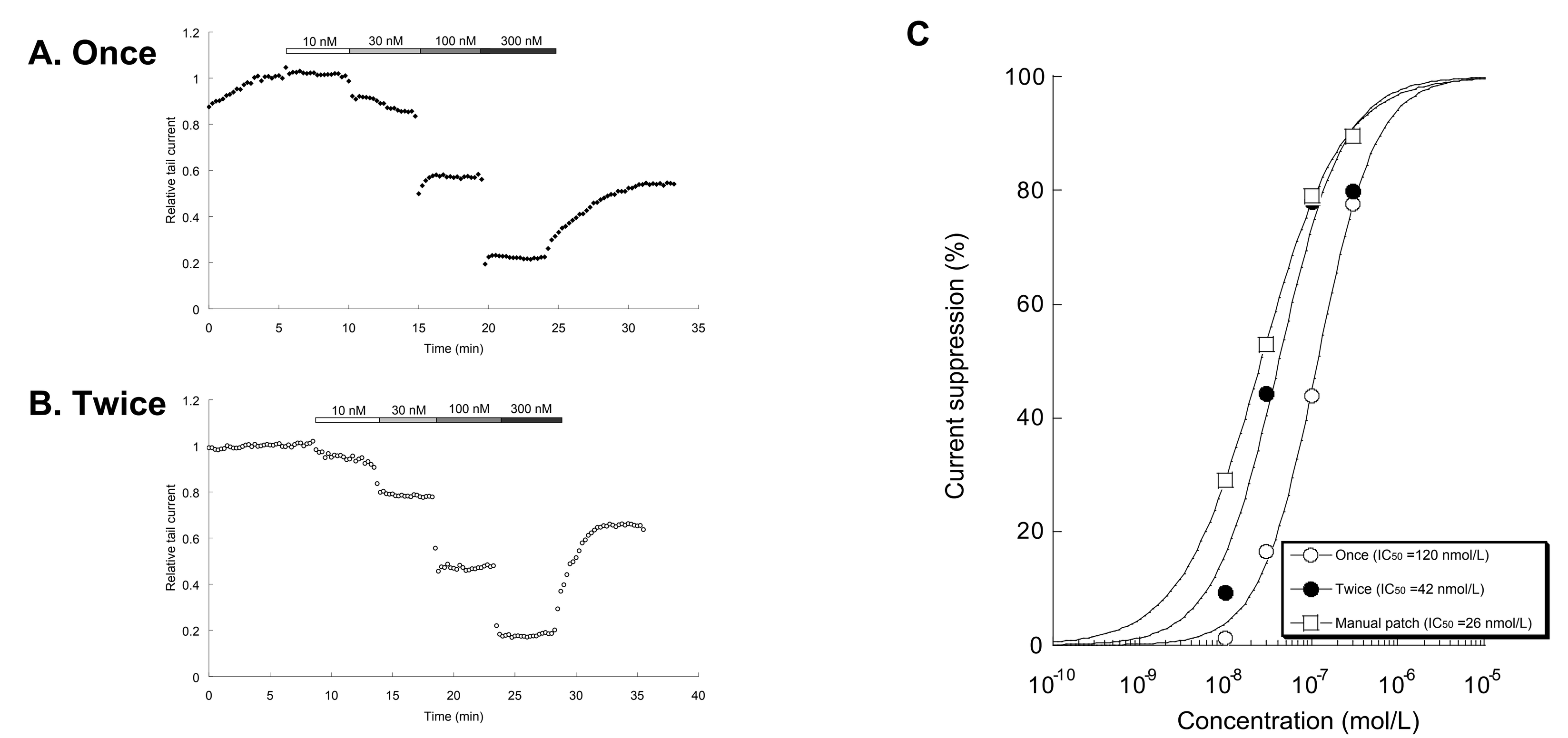


4. Result -Voltage Protocol Optimization-



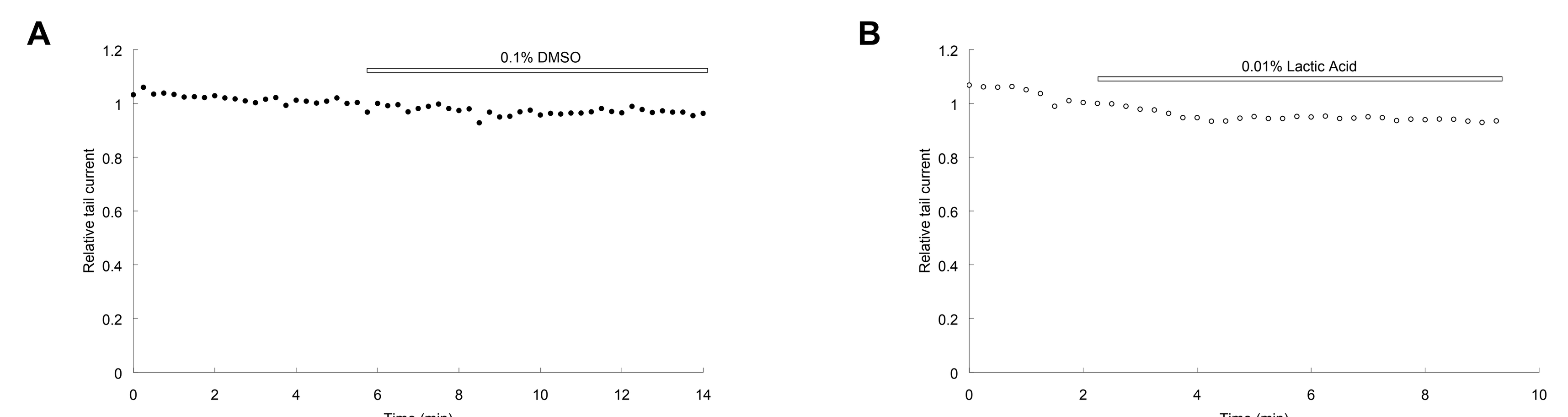
Optimization of voltage protocols for the automated patch-clamping. A; Schematic diagrams of voltage protocol used to optimize potential and duration for depolarization. The cell membrane of hERG-transfected HEK293 cell was held at -70 mV and depolarized to voltages from -50 to 40 mV for 0.75 to 2 sec, and then repolarized to -50 mV for 2 sec. B; Representative currents induced by the depolarizing step pluses. C; The current-voltage relationships for the hERG currents. The outward current at the end of the depolarizing step and the peak tail currents when the voltage protocol described in A was run were plotted as a function of the membrane potential of depolarizing pulse. D; Schematic diagrams of voltage protocol used to optimize potential for repolarization. The cell membrane was depolarized to 20 mV for 2 sec to activate and slightly inactivate the hERG potassium channels, then clamped to various level of potentials from -80 to 50 mV for 2 sec. E; Representative currents induced by the voltage protocol shown in D. F; The current-voltage relationships for the hERG currents. The peak tail currents when the voltage protocol described in D was run were plotted as a function of the membrane potential of repolarizing pulse.

5. Result -Application Procedure Optimization-



Optimization application procedure of test solutions to cells. A; Time course of the changes in relative tail current when haloperidol at concentrations of 10, 30, 100 and 300 nmol/L were cumulatively injected into recording chamber. The solution was changed only once in respective concentration. B; The same time course of the changes in relative tail current, but the solution was changed twice in respective concentration. C; The dose-response relationships of haloperidol block of the hERG current in the automated patch-clamping with once or twice injections and in the manual patch-clamping.

6. Result -Current Stability-



Effects of vehicles to dissolve test substances on the hERG current in the automated patch-clamp system. A; Dimethyl sulfoxide at 0.1% (v/v). B; Lactic acid at 0.01% (v/v).

7. Summary and Conclusion

- hERG channels were fully activated by depolarizing steps greater than 0 mV for 2 sec.
- Amplitude of peak tail current was maximized by the depolarizing pulse greater than 10 mV for 1.5 sec.
- Amplitude of peak tail current was maximized between -50 mV and -40 mV.
- An optimum voltage protocol was determined as follows;
  - Holding potential: -70 mV
  - Depolarizing potential: 20 mV
  - Depolarizing duration: >2 sec
  - Repolarizing potential: -50 mV
- Sequential exchange of extracellular solution for is recommended at twice for reproducible data generation.