High throughput hERG assay using the automated patch-clamp system; Comparison to conventional patch clamping

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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 part of Safety Pharmacology Studies to predict the potential risk of torsade de pointes in the clinical use of drugs. Since potassium currents passing though the hERG channels can be measured directly by patch-clamping, the experiment is considered to be more reliable than other kinds of experiments such as the rubidium efflux assay. However, the patch-clamp experiments are very time consuming and the throughput is much lower than those of other assays. In order to dramatically enhance the efficiency of patch-clamp experiments, 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 the effects of drugs on the hERG channel transfected in HEK 293 cells. Various voltage protocols to induce hERG currents were determined and the experimental conditions were optimized. Under these optimized conditions, the effects of standard drugs on the hERG channel could be assessed relevantly.
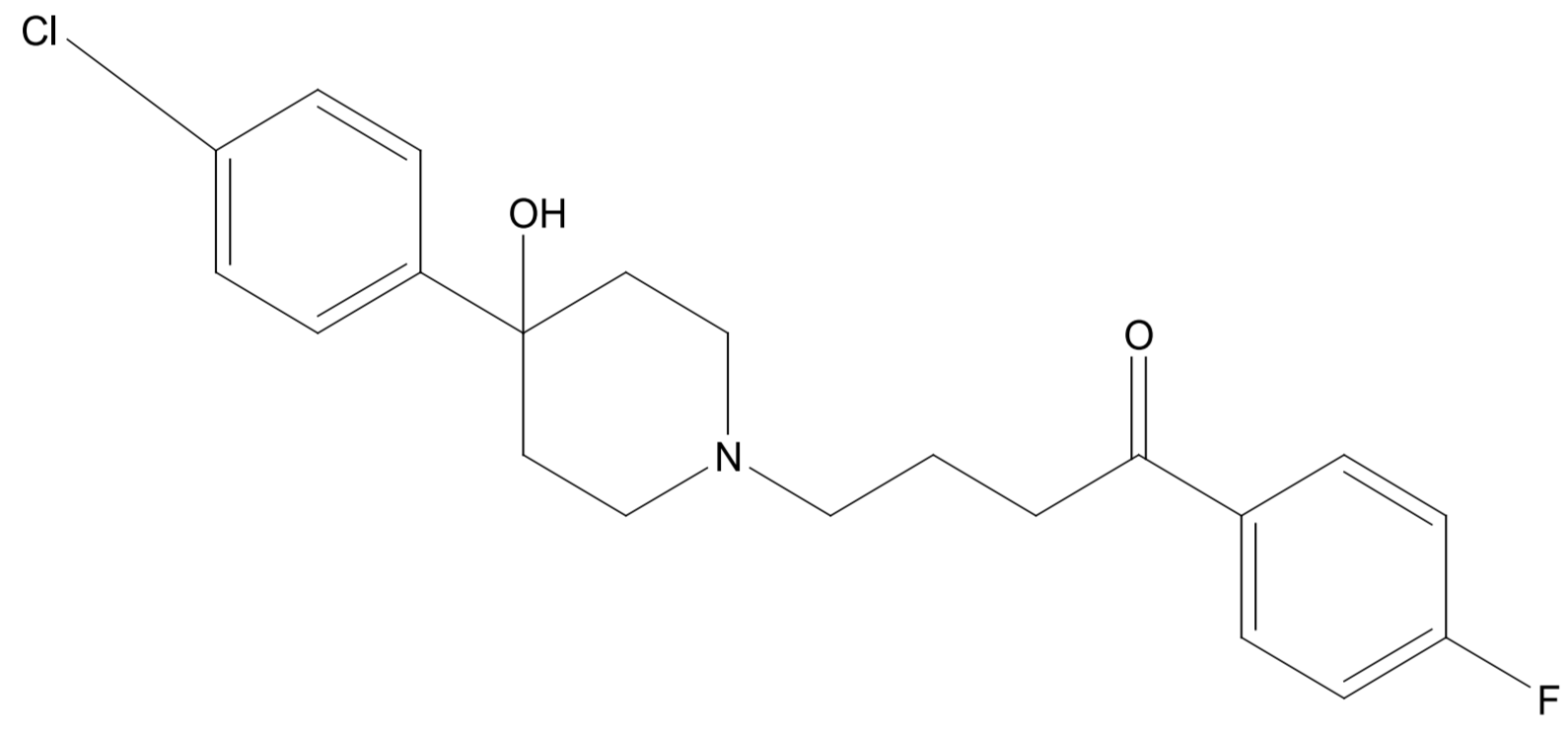
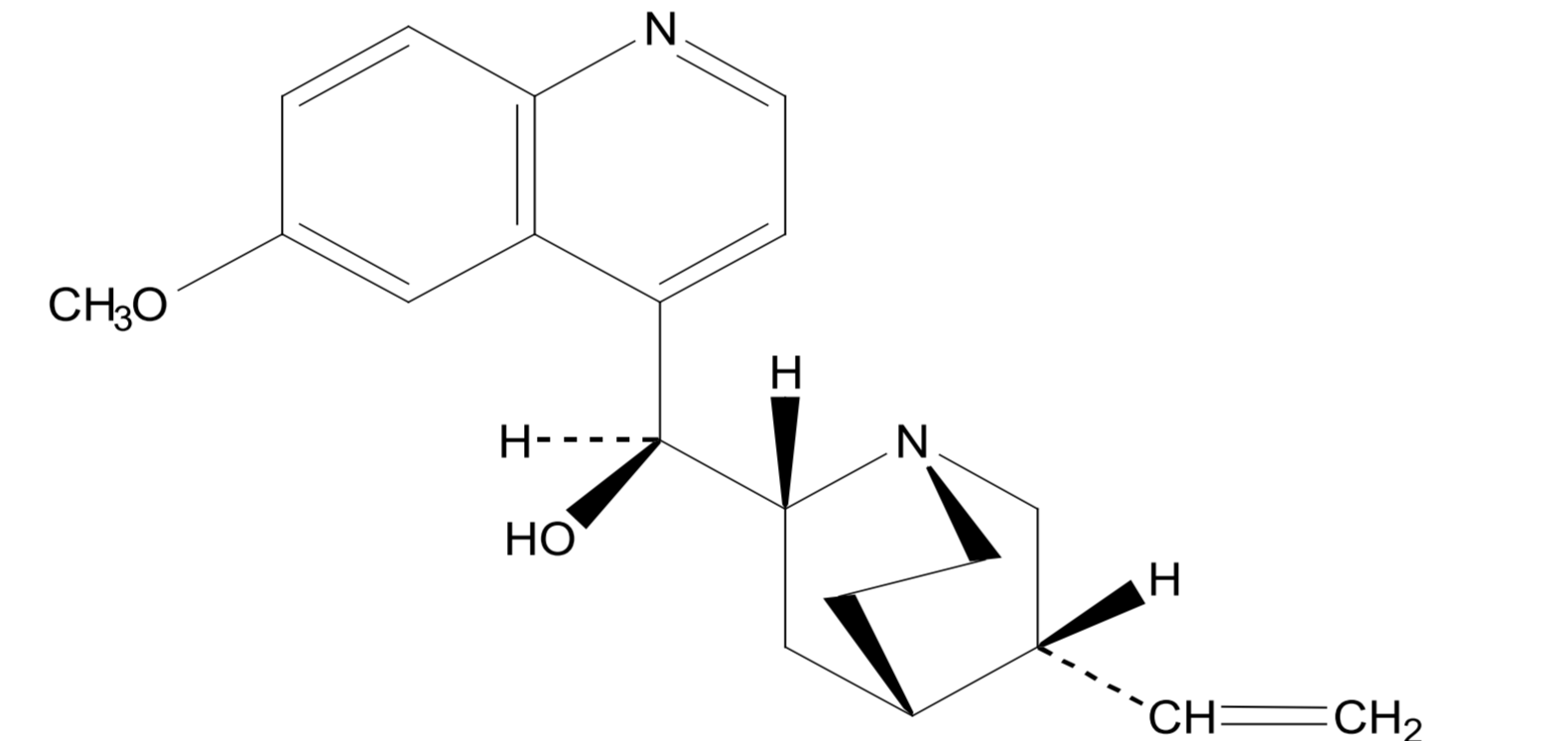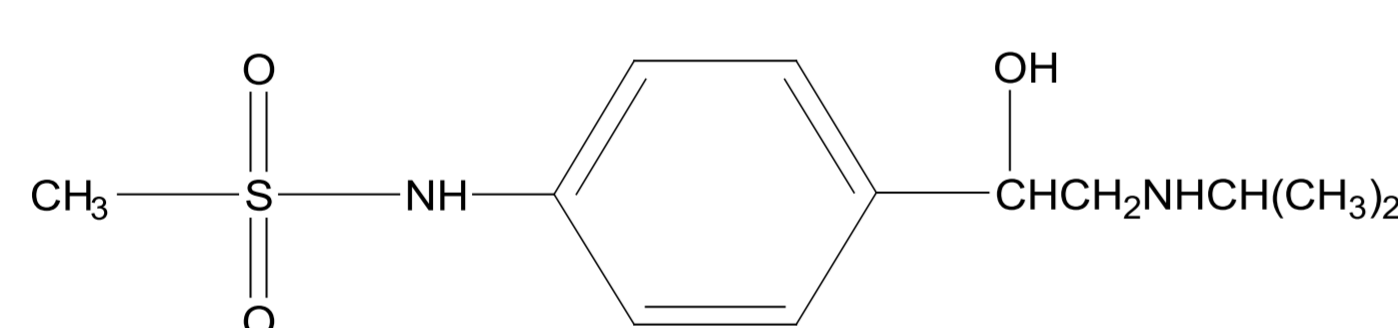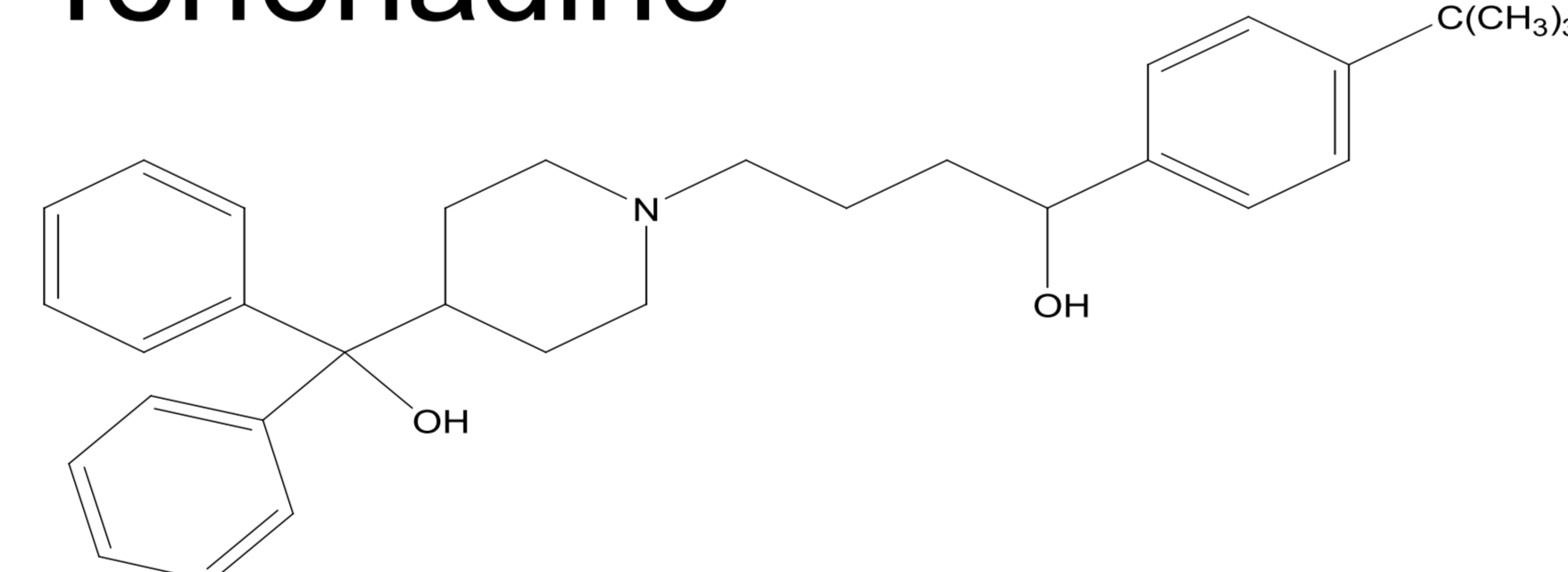## 2. Test substances

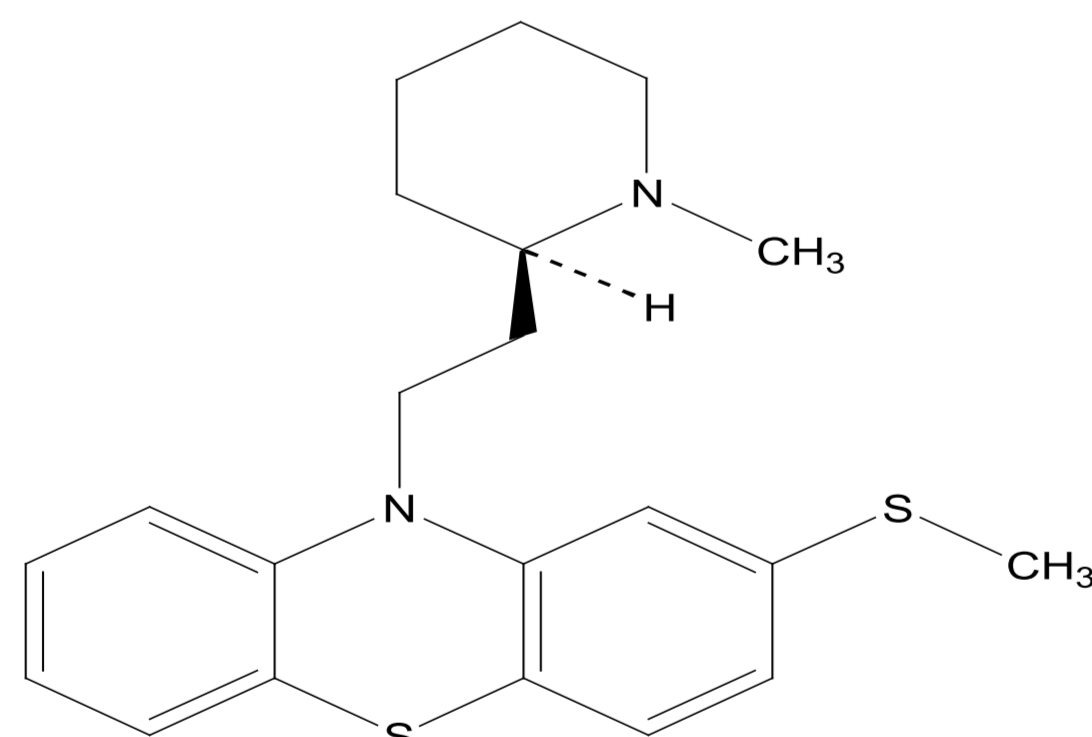A. Diphenhydramine
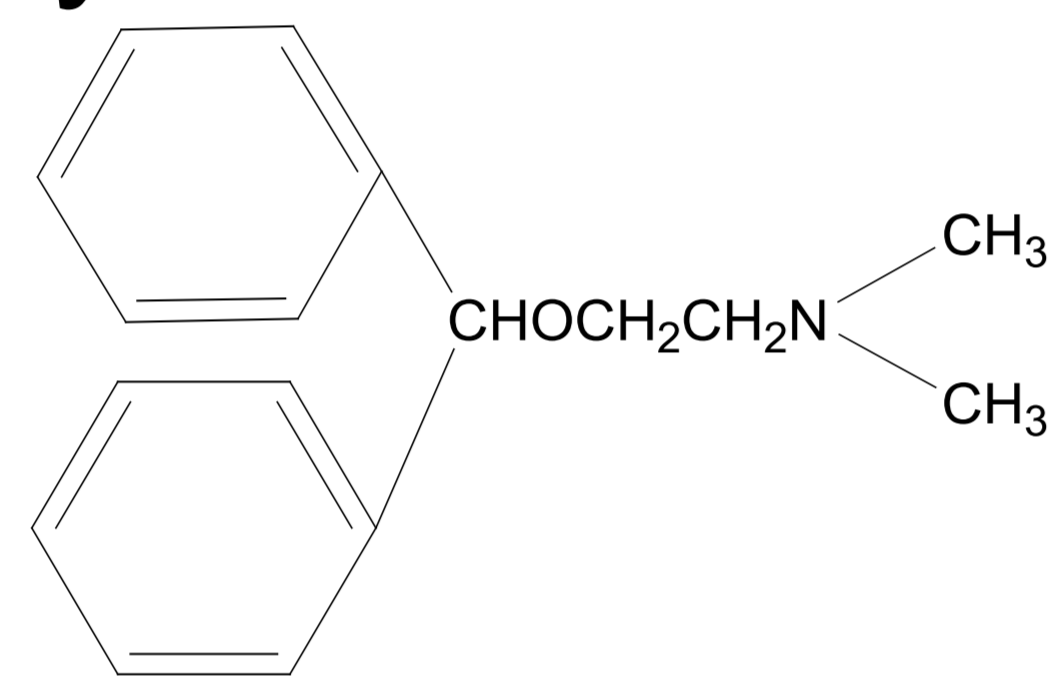
B. E-4031
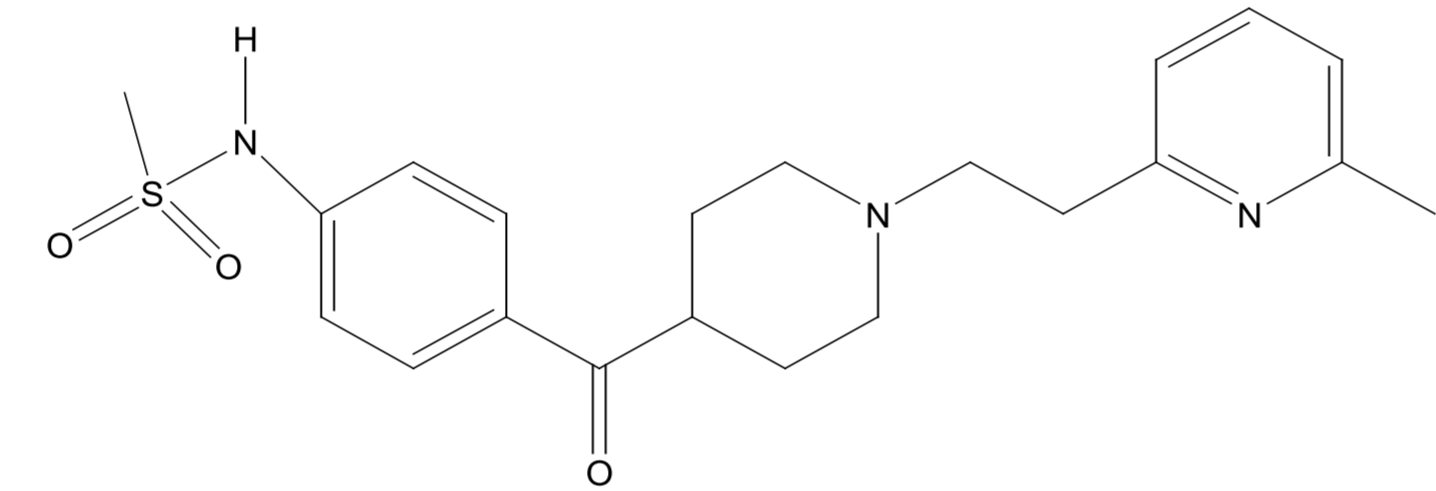
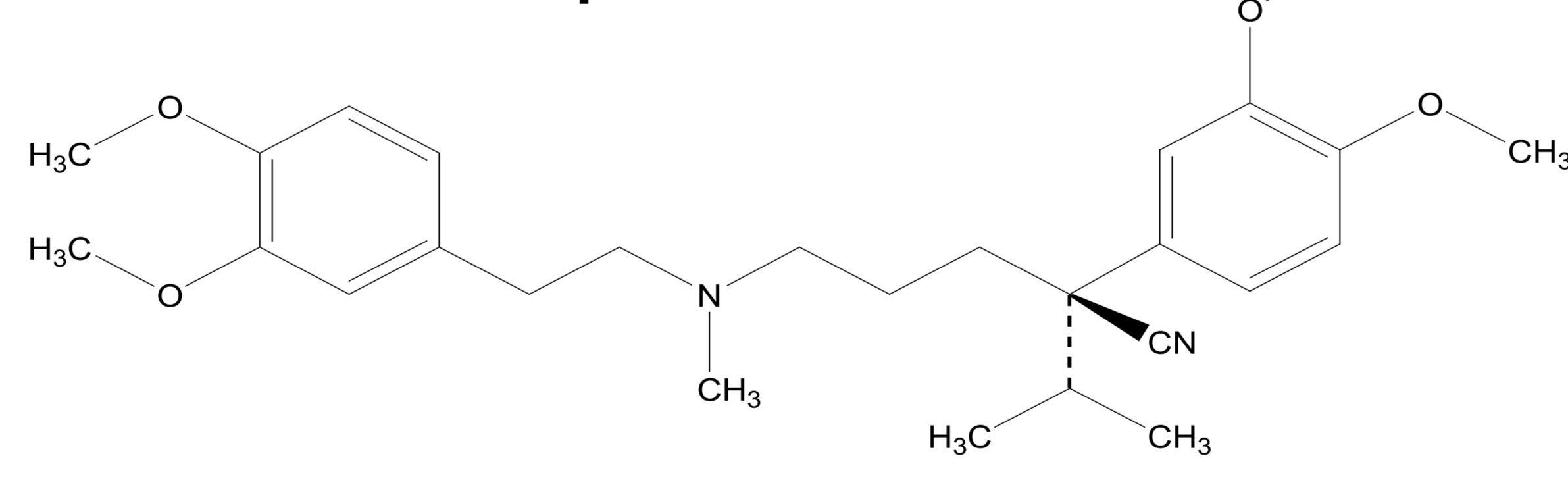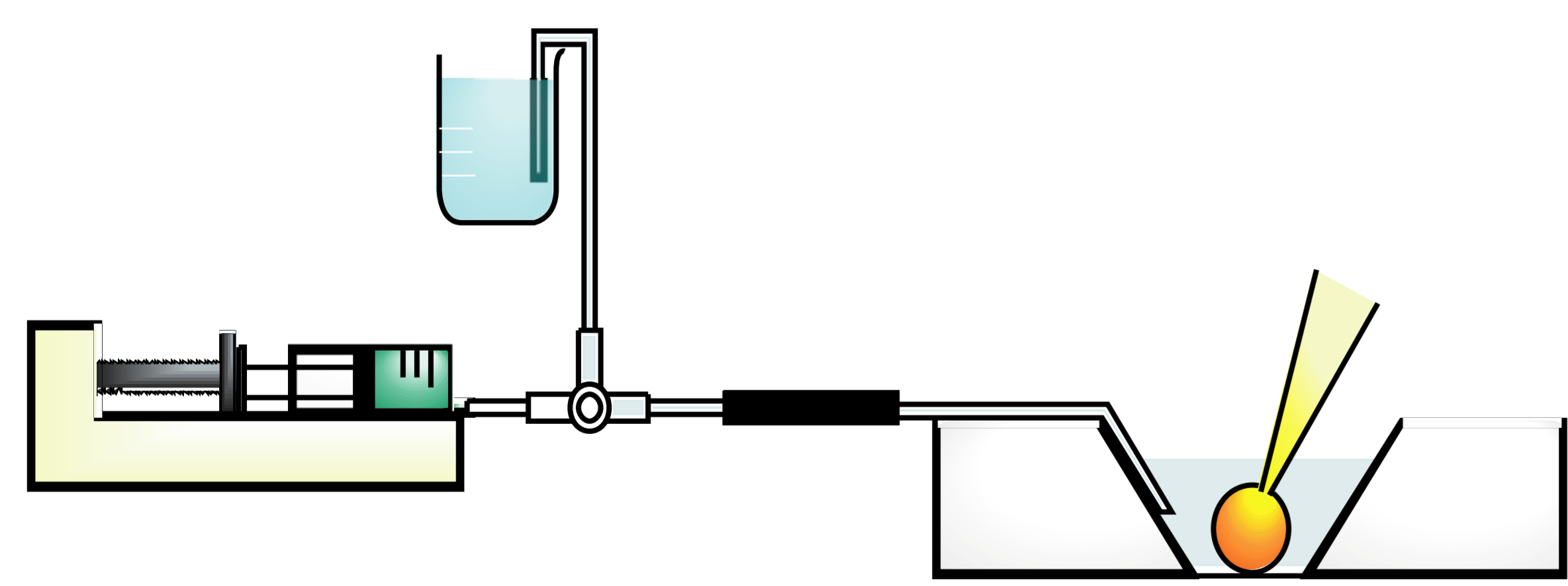C. Haloperidol

D. Quinidine

E. Sotalol
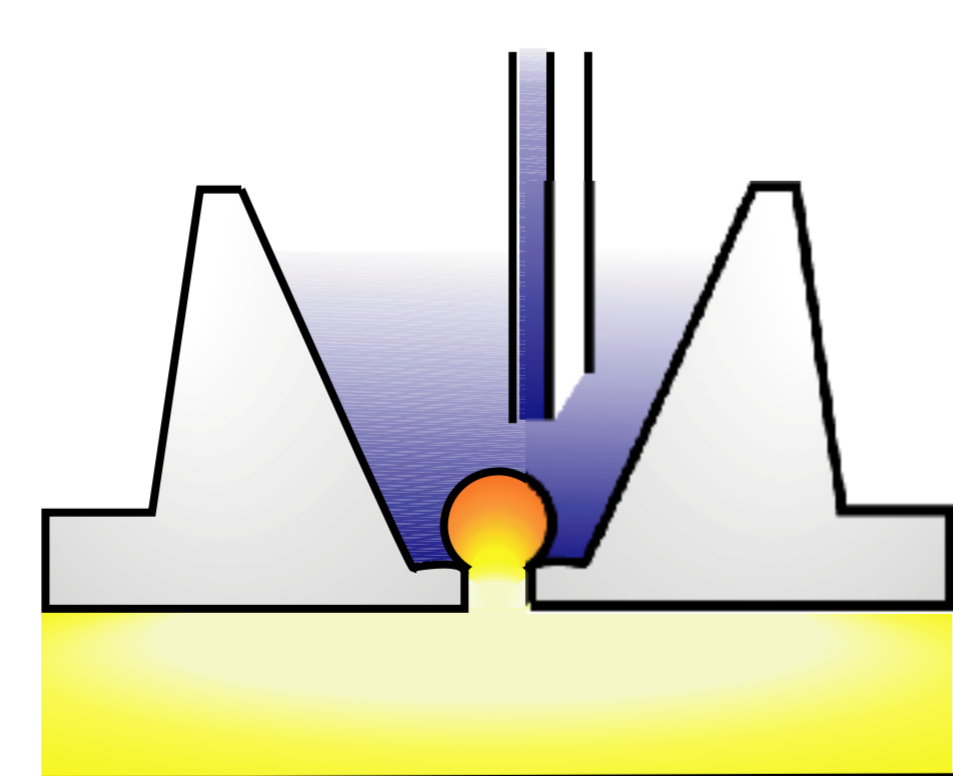
F. Terfenadine

G. Thioridazine

H. Verapamil

3. Methods
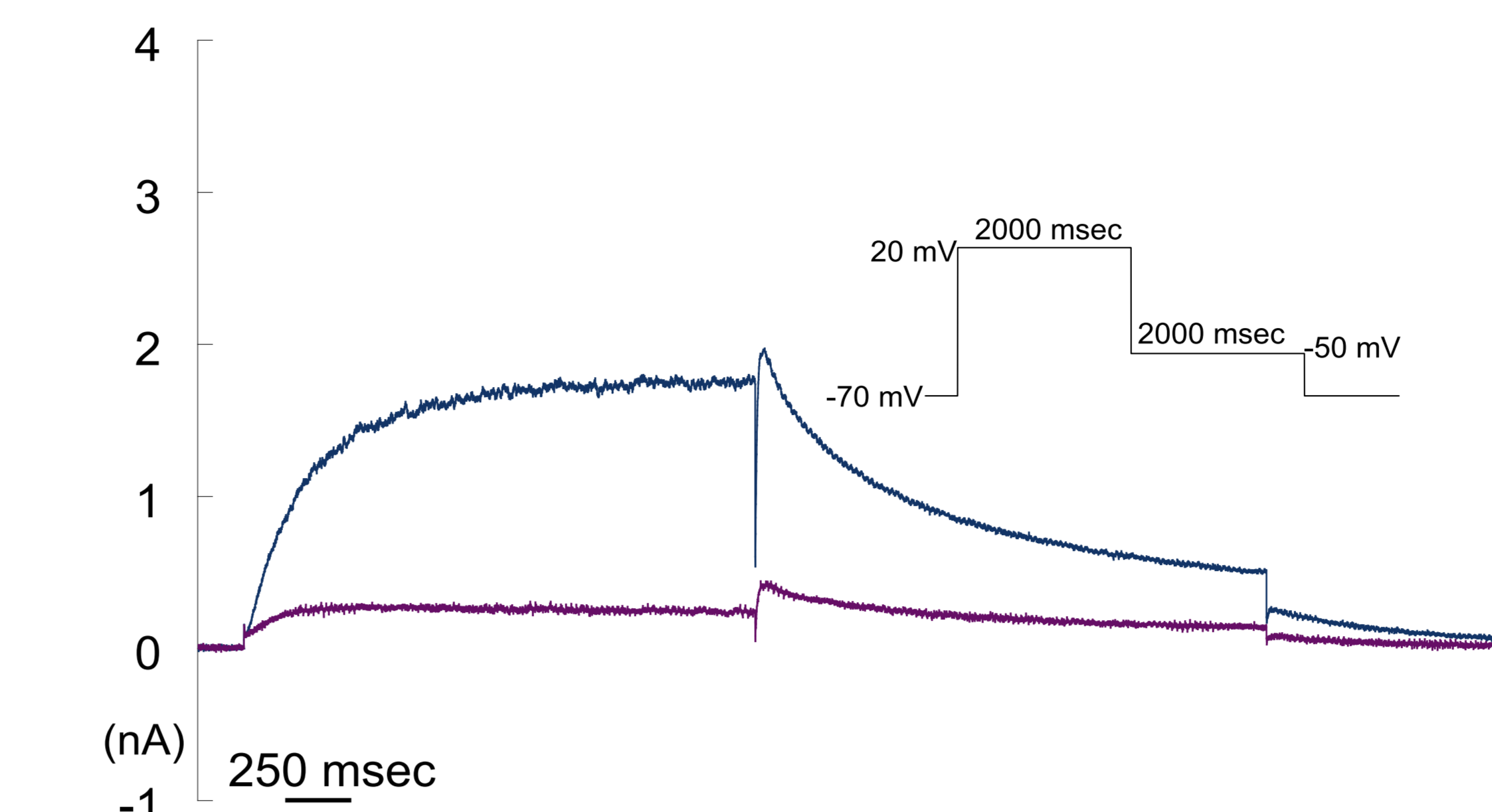
A. Conventional Patch-clamping
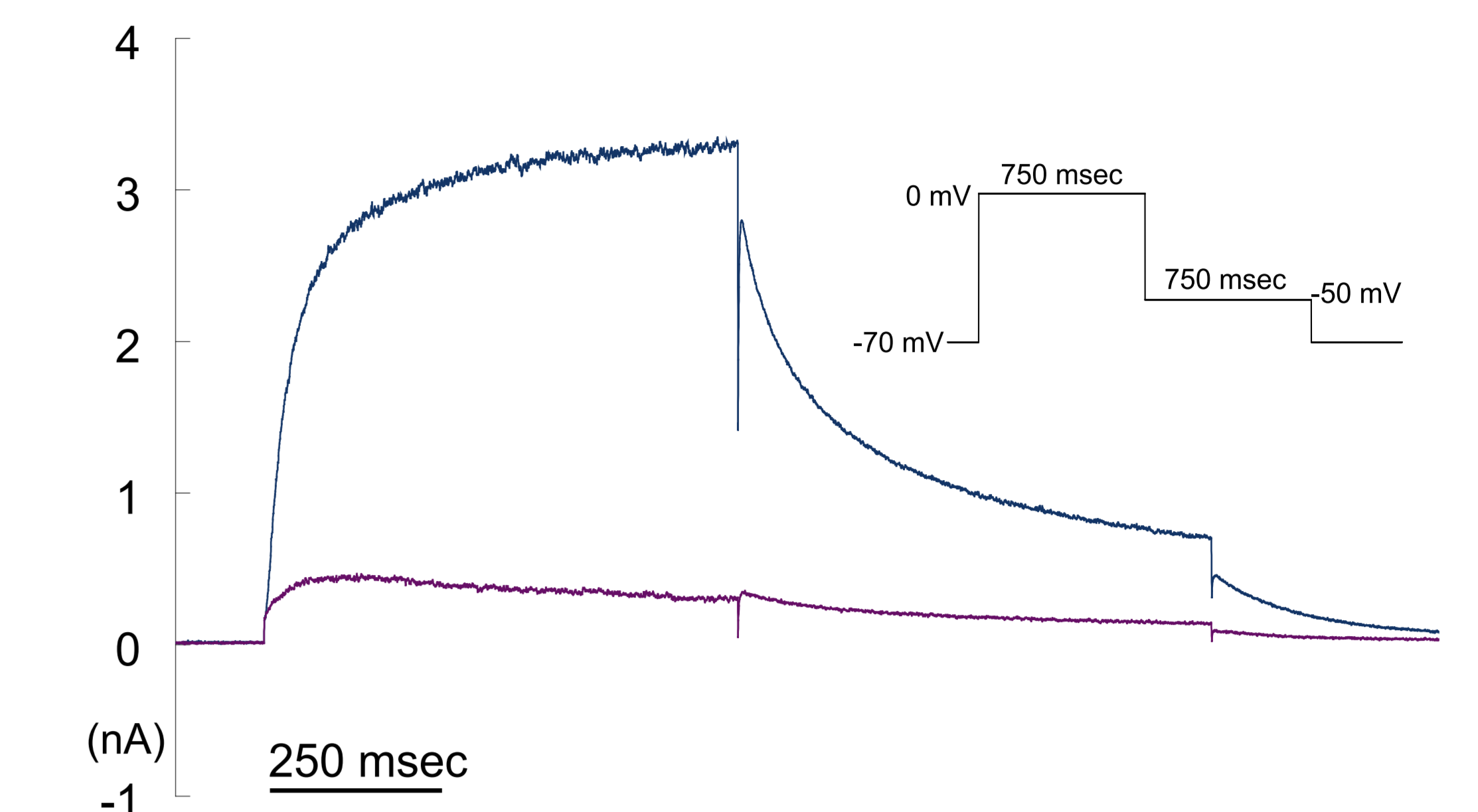
B. Automated Patch-clamping

Test methods employed to determine the suppressive effects of test substances on hERG potassium channels transfected in HEK293 cells. A; A schematic diagram of the test substance application in the conventional patch-clamping system. B; A schematic diagram of test substance application in the automated patch-clamping system.
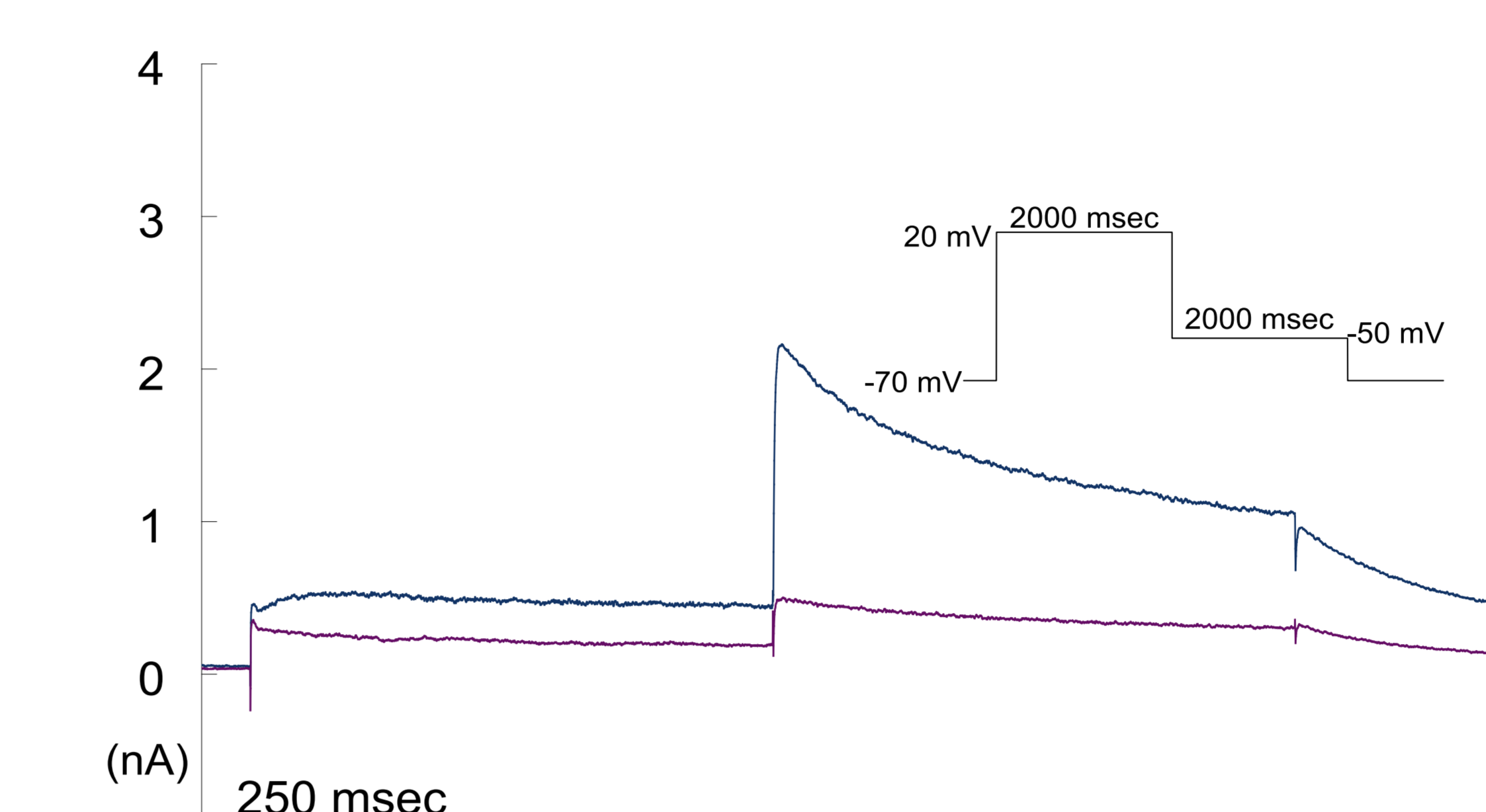
4. Effects of E-4031 on the hERG current
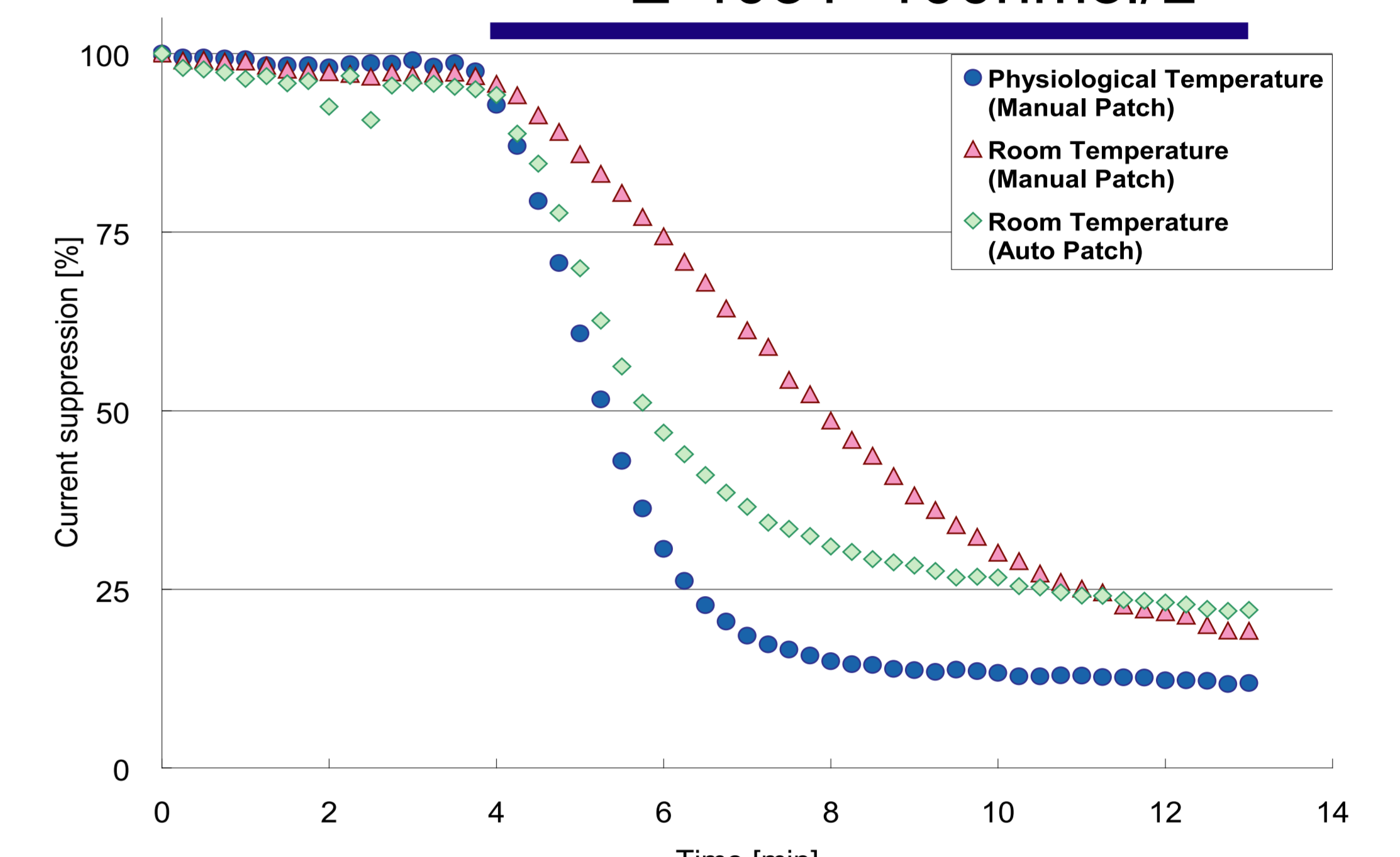
A. Conventional-Room Temp.

B. Conventional-Physiol. Temp.

C. Automated-Room Temp.

D. E-4031 100nmol/L

Effects of E-4031 on the hERG current in conventional and automated patch-clamping systems. A; The voltage clamp protocol and currents recorded from a hERG-transfected cell in conventional patch-clamping at room temperature. B; The voltage clamp protocol and currents recorded in conventional patch-clamping at $37\pm1°C$. C; The voltage clamp protocol and currents record in automated patch-clamping at room temperature. The time course of the changes in relative tail current when E-4031, at a concentration of 100 nmol/L, was injected into the recording chamber of conventional and automated patch-clamp system.

5. Effects of test substances on the hERG

A. Conventional-Physiol. Temp. B. Conventional-Room Temp. C. Automated-Room Temp.
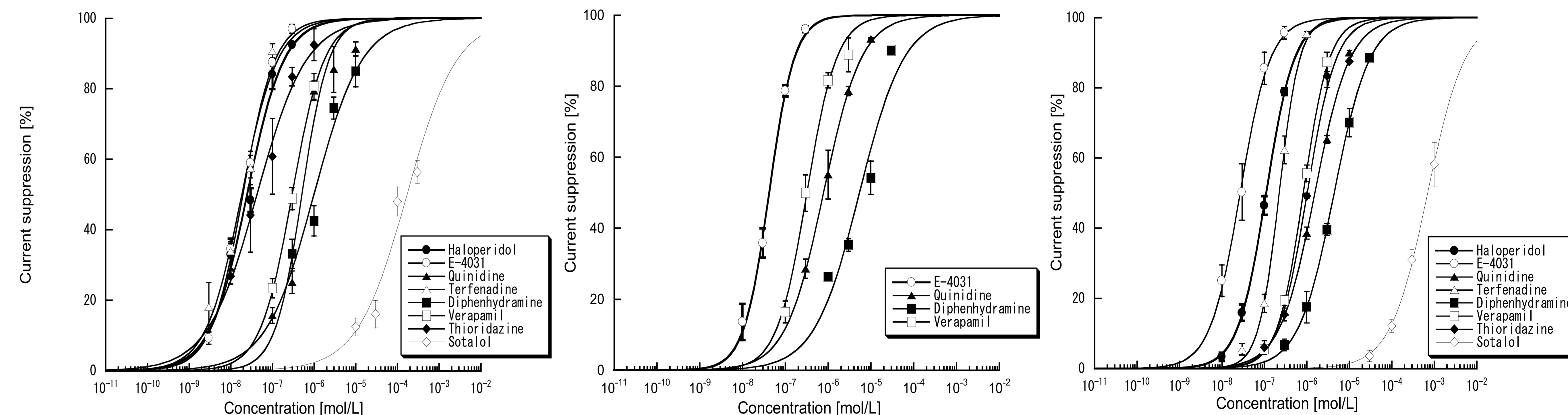
Concentration-response relationship of test substances on hERG potassium channel currents in HEK293 cells. The value of the current used to determine the effects of a test substance is the peak outward tail current amplitude repolarized at -50 mV following the initial depolarizing step to 0 or 20 mV from a holding potential of -70 mV. These pulses were given every 15 sec. The percent suppression of the hERG current is plotted as a function of the test substance concentration. Data is fitted using the Hill equation. Each point indicates the mean $\pm$ SEM (n=3 or 7). A; Data obtained in the conventional patch-claming at room temperature. B; Data obtained in conventional patch-claming at $37\pm1°C$. C; Data obtained in automated

6. Summary

Test substance	IC50 Value (nmol/L)		
	Manual Patch (Physiological Temperature)	Manual Patch (Room Temperature)	Autol Patch (Room Temperature)
Diphenhydramine	997	5434	4417
E-4031	20	42	27
Haloperidol	26	N.A.	110
Quinidine	514	788	1546
Sotalol	149860	N.A.	>100000
Terfenadine	18	N.A.	224
Thioridazine	43	N.A.	1024
Verapamil	299	321	830

7. Conclusion

■ The IC$_{50}$ values for the suppressive effects of test substances on the hERG channel obtained in the automated patch-clamp system tended to be higher than those obtained in conventional patch-clamping at physiological temperature.

■ The differences in IC$_{50}$ values between automated and conventional patch-clamping, at room temperature, using the same voltage protocol were smaller than those between automated patch-clamping at room temperature and the conventional patch-clamping at physiological temperature.

■ The temperature of extracellular solution seemed to be one of the factors that caused the difference in IC50 values between the test procedures, the saitomated and conventional patch-clamping.

■ Since the extracellular solution was not perfused in the automated patch-clamp system, the possibility that the could be adsorption of the test substances in the application system should also be considered as a factor responsible for the differences in IC50 values between test procedures automated and conventional patch-clamping.