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In vitro safety pharmacology assay using guinea pig cardiac myocyte

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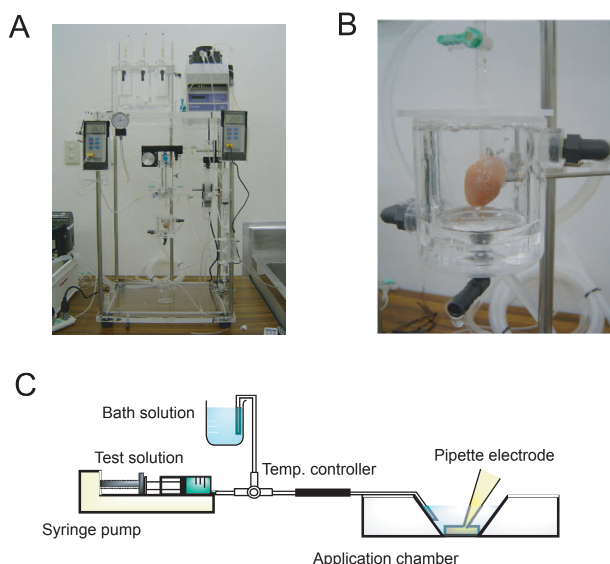
1. Introduction

In order to predict the drug-induced QT prolongation, a great diverse of assays has been developed recently. Among them, the *in vitro* QT screening of compounds at early stages of the drug discovery program to detect influences on the hERG current and/or cardiac action potential repolarization attract attention due to their reliability and facility. On the other hand, as an *in vitro* QT screening assay, the patch-clamp experiment using cardiac myocytes has advantages compared to other *in vitro* assays. Because, the effects of compounds on all ion channels underlying to exert cardiac action potentials can be investigated simultaneously and the complex interaction of the ion channels is well reflected in the assay. Since the guinea pig cardiac myocyte possesses most of ion channels that express in the human cardiac myocyte, it could be one of the most relevant test systems for the *in vitro* QT screening assay.

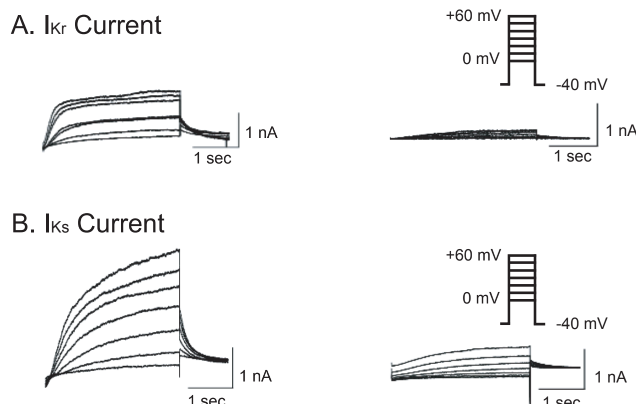
In this study, we examined two potassium channel blockers on the rapidly activating delayed rectifier K^+ current (I_{Kr}) or slowly activating delayed rectifier K^+ current (I_{Ks}) to investigate their electrophysiological actions in guinea pig cardiac myocytes using the whole-cell patch-clamp technique. Our results suggest that the isolated cardiac myocyte is one of very feasible test systems for predicting the drug-induced QT prolongation.

2. Materials and Methods



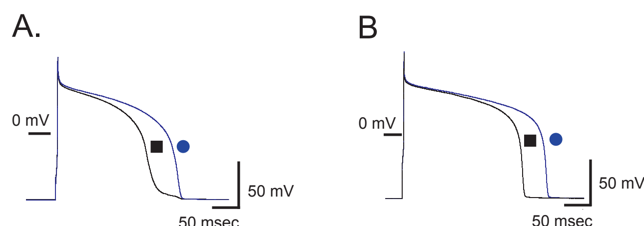
A: The entire picture of langendorff perfusion system. B: The langendorff apparatus to mount the heart. The guinea pigs were deeply anesthetized by pentobarbital sodium, and then their hearts were rapidly excised. The aorta was cannulated and mounted on the apparatus. The heart was retrogradely rinsed for 5-8 min with Tyrode's solution, consisting of NaCl 143, KCl 5.4, NaH_2PO_4 0.33, $MgCl_2$ 0.5, $CaCl_2$ 1.8, Glucose 5.5, HEPES 5 (in mmol/L, pH 7.4 was adjusted to by NaOH) and then perfused for 8-10 min with calcium-free Tyrode's solution. The heart was enzymatically digested by perfusing with calcium-free Tyrode's solution containing collagenase (0.2 mg/mL). After 15-20 min of digestion, the heart was perfused with KB solution, consisting of KOH 70, L-Glutamic acid 50, KCl 40, Taurine 20, KH_2PO_4 20, $MgCl_2$ 3, Glucose 10, EGTA 1, HEPES 10 (in mmol/L, pH was adjusted to 7.4 by KOH). The temperature of these perfusates was maintained at 37℃ and equilibrated with O_2. The heart was then minced in KB solution to disperse cells, and then kept at 4℃. C: The schematic diagram of drug application system. Whole-cell currents and action potentials were recorded from isolated cardiac myocytes using patch-clamp technique. The test solution was superfused into the application chamber using a syringe pump (KDS200, KD Scientific Inc.). A temperature controller (TC-344B, Warner Instruments, LLC) was used to monitor and control the temperature of the Tyrode's solution in the application chamber at 37.0℃±1.0℃. The pipette solution was composed of the following (in mmol/L): KCl 130, $MgCl_2$ 1, EGTA 5, MgATP 5, HEPES 10 (pH was adjusted to 7.2 by KOH). Pipette electrodes were made of borosilicate-glass capillaries (G-1.5, Narishige Scientific Instrument Lab.) using a puller (P-97, Sutter Instrument Co.). Pipette electrodes with a resistance of 2.0-5.0 mega-ohms were used. Whole-cell currents and action potentials were measured with an amplifier (Axopatch-200B, Molecular Devices Corporation), and electric signals were recorded onto a computer hard drive using software (pCLAMP 9, Molecular Devices Corporation).

3. Effects of K^+ Channel Blockers on the I_{Kr} and I_{Ks} Channel



Effects of potassium channel blockers on the I_{Kr} or I_{Ks} channel. A: Effects of E-4031 at a concentration of 10 μM on I_{Kr} channels in guinea pig ventricular myocyte. The voltage protocol described in the inset was run. The cell membrane was held at -40 mV, depolarized to voltages ranged from 0 to 60 mV for 3 seconds, and then repolarized to -40 mV. The current traces after application of 10 μM E-4031 are shown on the right. B: Effects of chromanol 293B at a concentration of 30 μM on I_{Ks} channels in guinea pig ventricular myocyte. The voltage protocol described in the inset was run. The cell membrane was held at -40 mV, depolarized to voltages ranged from 0 to 60 mV for 3 seconds, and then repolarized to -40 mV. The current traces after application of 30 μM chromanol 293B are shown on the right.

4. Effects of K^+ Channel Blockers on APD



Effects of potassium channel blockers on action potential duration (APD). A: Effect of E-4031 at a concentration of 10 μM on APD in guinea pig ventricular myocyte. Action potentials were evoked by applying a single pulse at a frequency of 0.2 Hz. Action potentials under control condition (■) and after 5-minute superfusion with 10 μM E-4031 (●) were superimposed. B: Effect of chromanol 293B at a concentration of 30 μM on APD in guinea pig ventricular myocyte. Action potentials were evoked by applying a single pulse at a frequency of 0.2 Hz. Action potentials under control condition (■) and after 5-minute superfusion with 30 μM chromanol 293B (●) were superimposed.

5. Conclusion

In this study, E-4031 and chromanol 293B dominantly suppressed IKr and IKs isolated from the whole-cell current in guinea pig ventricular myocyte, respectively. The potassium current inhibitors remarkably prolonged APD in guinea pig ventricular myocyte as well. These results indicate that the patch-clamp experiments under voltage as well as current clamp conditions using isolated cardiac myocyte are useful for predicting the drug-induced QT prolongation.

6. Acknowledgments

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