The reliability of patch-clamp experiments using guinea pig cardiac myocyte in safety pharmacology

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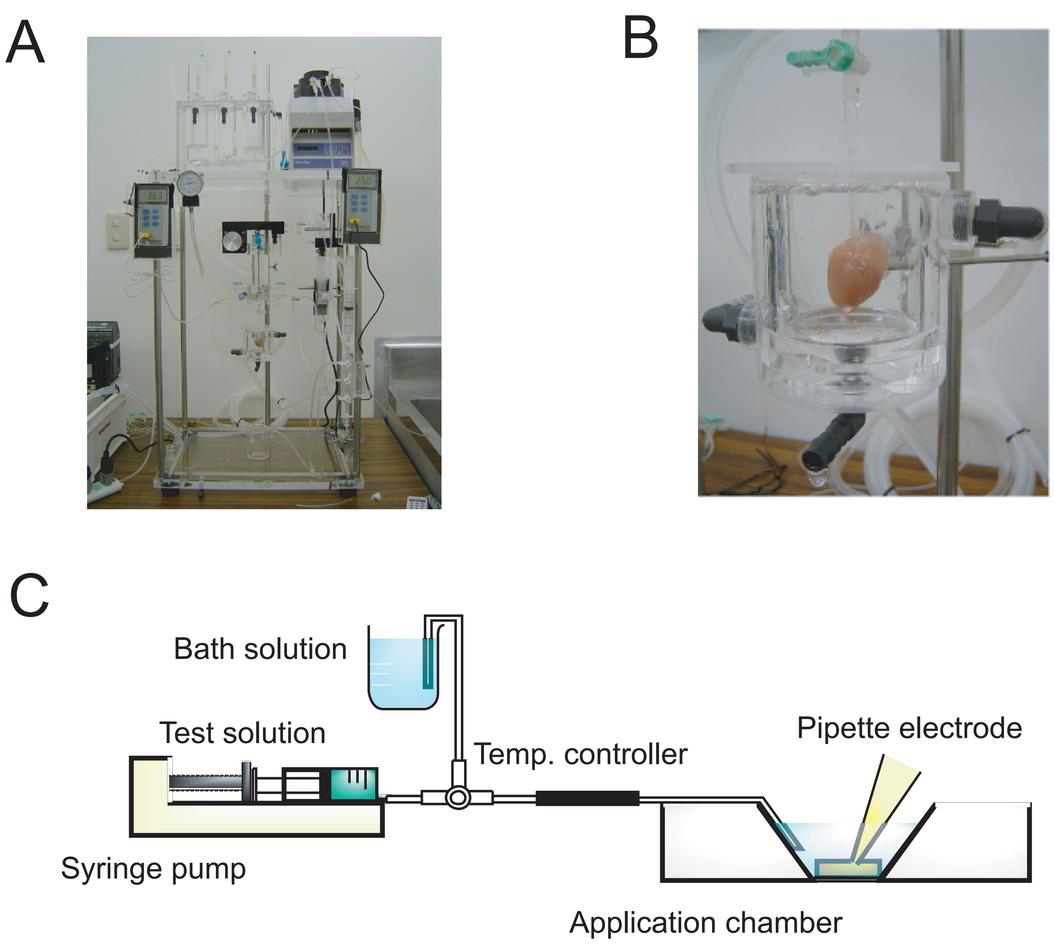
1. Introduction

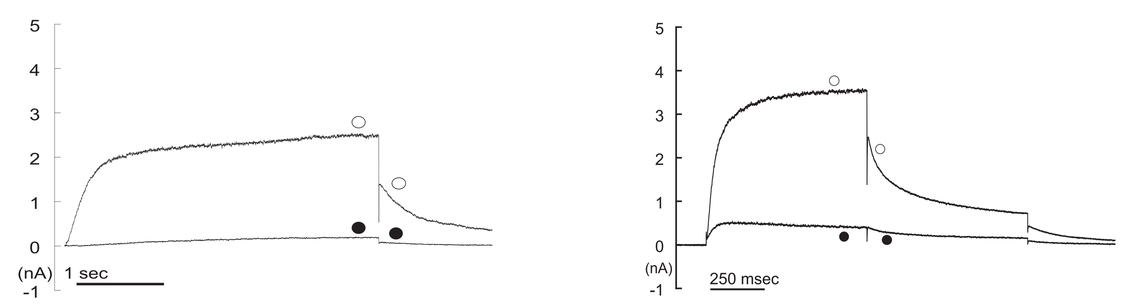
In order to predict the drug-induced QT prolongation, a great diverse of assay has been developed recently. Among them, the IKr assay using a recombinant cell line that expresses hERG channels and/or the repolarization assay using cardiac muscles are widely implemented as an in vitro electrophysiological study today. The patch-clamp experiment using cardiac myocytes, employed in this study, has advantages when 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 E-4031 which is well known to prolong QT intervals to investigate its electrophysiological actions, and investigated correlations between the whole-cell patch-clamp technique using guinea pig cardiac myocytes or hERG transfected cells using and the microelectrode technique using guinea pig papillary muscles.

2. Materials and Methods



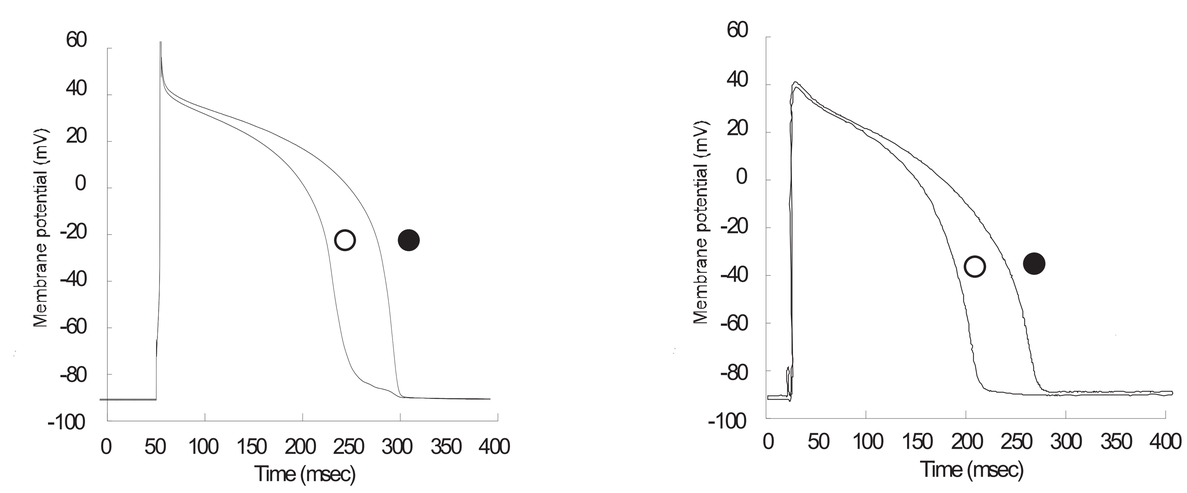
Test methods employed to determine the suppressive effects of test substances. A: The entire picture of langendorff perfusion system. B: The langendorff apparatus to mount the heart. C: The schematic diagram of drug application system. Whole-cell currents and action potentials were recorded from isolated cardiac myocytes or hERG transfected cells using patch-clamp technique. D: A schematic diagram of the test substance application system for action potential duration assay using guinea pig papillary muscles.

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



Effects of E-4031 on the I_{Kr} or hERG channels. A: Effects of E-4031 at a concentration of 10 μM on I_{Kr} channels in guinea pig ventricular myocyte. The cell membrane was held at -40 mV, depolarized to voltages to 50 mV for 3 seconds, and then repolarized to -40 mV. E-4031 at 10 μM was applied to the cell for 10 min. B: Effects of E-4031 at a concentration of 100 nM on hERG transfected cell. The cell was held at -70 mV and depolarized to 0 mV for 0.75 sec to activate and slightly inactivate the hERG potassium channels, and then repolarized to -50 mV for 0.75 sec to induce the tail current. E-4031 at 100 nM was applied to the cell for 10 min.

4. Effects of K^+ Channel Blockers on APD



Effects of E-4031 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: Action potentials recorded from an isolated guinea pig papillary muscle. Electric stimuli were delivered to the sample muscle at a frequency of 1 Hz using a stimulator and an isolator. Action potentials under control condition and after 30-minute superfusion with 100 nM E-4031 were superimposed.

5. Conclusion

In this study, E-4031 suppressed I_{Kr} isolated from the whole-cell current in guinea pig ventricular myocyte. E-4031 remarkably prolonged APD in guinea pig ventricular myocyte as well. These results corresponded to the results of patch-clamp assay using hERG transfected cells and microelectrode assay using guinea pig papillary muscle.
Based on the results, it is indicate that the isolated cardiac myocyte is one of the very feasible test systems for predicting the drug-induced QT prolongation. Further studies are underway to determine effects of drugs for the quantitative analysis.