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Optimization of test procedures for the cumulative application of test substance in APD study in isolated guinea-pig ventricular papillary muscles

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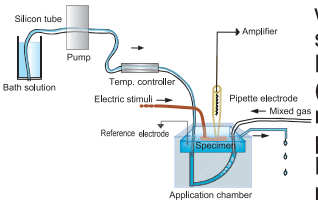
1. Introduction

The action potential (AP) assay using guinea-pig papillary muscles has been used widely as a part of Safety Pharmacology Studies in order to detect potency of test articles for QT prolongation in the electrocardiogram. As one of issues in the AP assay, the reduction of number of animals used for preparing papillary-muscle samples is important from the point of view of the animal protection. For this purpose, the cumulative application of test articles is recommended. However, the AP need to be stabilized for considerably long duration to complete the multiple applications, so that the success rate of the experiment tends to decline compared to the single-dose application. This study was conducted to investigate parameters, especially the stimulus frequency, that may affect the stability of AP.

2. Materials and Methods



Action potentials were recorded using an isolated guinea-pig papillary muscle. Electric stimuli (voltage: 2-times higher than the threshold, pulse length: 1 msec) were delivered to the sample muscle at a frequency of 1 or 0.5 Hz; the test solution was applied onto the sample for 30 minutes at a flow rate of 5 mL/min. Recorded action potentials were analyzed for the resting membrane potential (RMP) and action-potential duration (APD_{30}, APD_{60} and APD_{90}) before and 30 minutes after beginning of application. APD_{30}, APD_{60} and APD_{90} represent time periods for 30%, 60% and 90% repolarization of the action potentials, respectively. Furthermore, the difference between action-potential duration at 90% and 30% repolarization was calculated as $APD_{30\text{-}90}$. $APD_{30\text{-}60}$ and $APD_{60\text{-}90}$ were obtained in the same manner. The effects of test articles on the parameters are shown as percent changes from the preapplication value.

3. Result - Stability of Specimen

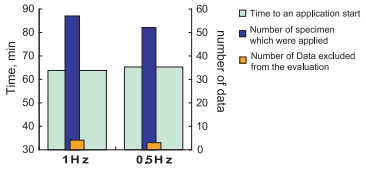


Fig. 1 Stability of specimen in different stimulation frequency.
The duration from isolation of a specimen to applying test solutions are shown. The number of recordings that were discontinued due to disconnection of a pipette electrode from a specimen were counted and compared with the number of the total data.

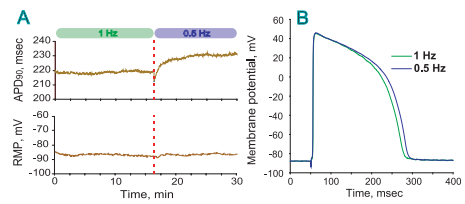


Fig. 2 APD in different stimulus frequencies.
A: Stimulus frequency was changed to 0.5 Hz from 1 Hz after the specimen was stabilized. APD_{90} and RMP are shown in the upper and lower charts, respectively. B: Waveforms of action potential when a specimen was stimulated at 1Hz or 0.5Hz.

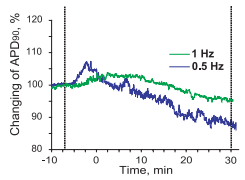


Fig. 3 Time course of change in APD_{90} with quinidine application
A APD_{30} values were normalized by taking the respective value at starting application (first dashed line) as 100%, and plotted as a function of duration (min). The recording was done for 30 minutes from the beginning of application.

4. Result - Frequency Dependence

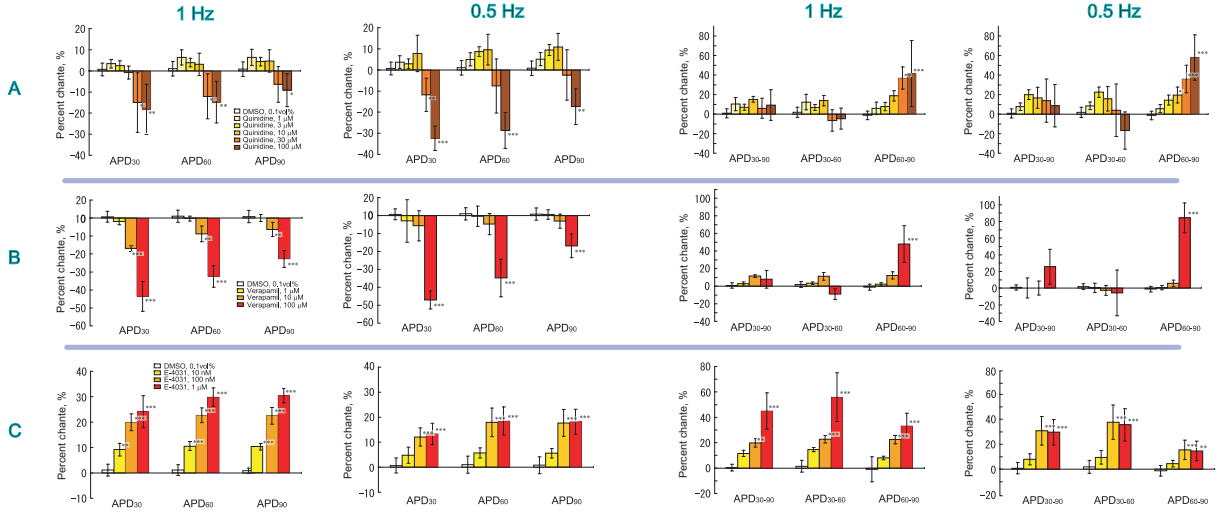


Fig. 4 Effects of test subustances on APD changed in stimulus frequency.
The dose response of test articles in APD and the difference of APDs in isolated guinea-pig ventricular papillary muscles stimulated at 1 Hz and 0.5 Hz are shown in the histograms. Each column represents the mean and error bars indicate SD (n=5 or 7). *, **, and ***: Significant difference at P<0.05, P<0.01, and 0.001, respectively, DMSO vs. test article, Dunnett's multiple test (parametric). A: Effects of quinidine . B: Effects of verapamil . C: Effects of E-4031.

5. Conclusion

Although it was anticipated that the lower stimulus frequency might contribute to a low rate of disconnection of a pipette electrode from a specimen, then result in better stability of AP and efficiency of the assay, no practical effect was observed (Fig.1). The APD elongated at the lower stimulus frequency of 0.5 Hz (Fig.2). This might be caused by enough recovery from inactivation of sodium and calcium channels between the longer stimulation intervals. When quinidine was applied, the test article exerted its effects in shorter time course at 0.5 Hz compared to those at 1 Hz (Fig.3). However in verapamil and E-4031, the time course of change was not affected. Furthermore, there was no difference between the stimulus frequencies in terms of the detection capability of effects of these test articles (Fig.4).

From these results, it was revealed that the modification of stimulus frequency does not contribute to the stability of a specimen and efficiency of the assay. Further studies to investigate other parameters, such as composition of Tyrode solution, shape of a chamber, and a flow rate of Tyrode solution, are required in order to optimize the AP assay in future.