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Short Communication

Developmental Changes in Action Potential Prolongation by K^+-Channel Blockers in Chick Myocardium

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Abstract. The effects of K^+-channel blockers on the action potential duration of the myocardium were examined in isolated right ventricles from the 7 – 10-day-old, 11 – 13-day-old, and 14 – 20-day-old embryo and 1 – 7-day-old hatched chicks. E-4031 significantly prolonged action potential duration at all developmental stages examined; the prolongation was largest in the 11 – 13-day-old embryo and was accompanied by early after-depolarizations. Chromanol 293B showed smaller prolongation at all stages examined. Terfenadine prolonged action potential duration in the 11 – 13-day-old embryo, but not in other stages. Thus, the chick ventricular myocardium changes its repolarization properties during development.

Keywords: chick, K^+-channel blocker, action potential duration

The chick embryo heart has been used to study various aspects of myocardial excitation and contraction. Although the chick heart shares common basic mechanisms with the mammalian heart, characteristic features in the electrophysiological properties, Ca^{2+} handling, contraction, and their physiological and pharmacological regulation has been reported (1 – 4). Concerning its electrophysiological properties, the resting membrane potential and action potential amplitude was reported to increase progressively during embryonic development (1), and many reports have been published concerning the depolarizing membrane currents involved (2). On the other hand, relatively little is known about its repolarization process, which is the determinant of the action potential duration. In the case of mammalian myocardia, information on the species difference and developmental changes in repolarization properties have accumulated, which enables appropriate selection of preparations for different experimental purposes (4). In the present study, we intended to obtain information about the repolarization mechanisms of the chick heart. We applied standard microelectrode techniques to the chick embryonic ventricular myocardia from four different developmental stages and examined the effect of inhibitors of the delayed rectifier potassium current, the major repolarizing current in the myocardium.

Standard microelectrode experiments were performed as previously described (5 – 7). Briefly, fertilized chicken eggs were incubated at 37°C, and the right ventricular free wall was isolated from 7 – 10-day-old embryo, 11 – 13-day-old embryo, 14 – 20-day-old embryo, and 1 – 7-day-old hatched chicks. They were driven at 1 Hz in an organ bath containing physiological salt solution of the following composition: 118.4 mM NaCl, 4.7 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM $MgCl_2$, 1.2 mM KH_2PO_4, 24.9 mM $NaHCO_3$, and 11.1 mM glucose. The solution was gassed with 95% O_2 – 5% CO_2 and maintained at 37°C (pH 7.4). All experimental data are expressed as the mean ± S.E.M., and statistical significance of differences between means were evaluated by the paired *t*-test. A *P* value less than 0.05 were considered significant. The drugs used were E-4031 (Wako, Osaka), chromanol 293B (Sigma, St. Louis, MO, USA), and terfenadine (Wako). All other drugs and chemicals were commercial products of the highest available quality.

The resting potential, overshoot and the maximum rate of rise increased progressively during the embryonic period (Table 1, Fig. 1A). Action potential duration increased progressively during the embryonic period. The action potential duration of 1 – 7-day-old hatched chicks

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Table 1. Developmental changes in action potential parameters

Parameters (units)	7 – 10-day-old embryo	11 – 13-day-old embryo	14 – 20-day-old embryo	1 – 7-day-old hatched chicks
RP (mV)	−78.8 ± 0.3	−80.8 ± 0.4	−82.4 ± 0.3	−82.8 ± 0.3
OS (mV)	34.4 ± 0.5	36.7 ± 0.4	37.7 ± 0.5	37.3 ± 0.3
APD_{20} (ms)	103.1 ± 2.7	103.4 ± 2.8	106.9 ± 2.9	102.3 ± 2.7
APD_{50} (ms)	152.8 ± 3.0	154.3 ± 2.7	163.9 ± 2.6	145.5 ± 2.8
APD_{90} (ms)	180.4 ± 2.6	185.6 ± 2.6	197.8 ± 1.7	168.5 ± 2.8
$\dot{V}_{max}$ (V/s)	118.2 ± 6.3	149.3 ± 8.4	180.1 ± 4.1	177.8 ± 3.7

Action potential parameters of ventricular myocardia from 7 – 10-day-old embryos, 11 – 13-day-old embryos, 14 – 20-day-old embryos, and 1 – 7-day-old hatched chicks were obtained in the absence of agents. RP, OS, and $\dot{V}_{max}$ indicate resting potential, overshoot, and maximum rate of phase-0 depolarization, respectively. APD_{20}, APD_{50}, and APD_{90} indicate action potential duration at 20%, 50%, and 90% repolarization, respectively. Each value is the mean ± S.E.M. from 36 experiments.

was shorter than that of the 14 – 20-day-old embryos.

E-4031 (1 μM), a blocker of I_{Kr}, significantly prolonged action potential duration at all developmental stages (Fig. 1: A, Ca). The magnitude of prolongation by E-4031 was larger in 7 – 10-day-old embryo and 11 – 13-day-old embryo than in the 14 – 20-day-old embryo and 1 – 7-day-old hatched chick. In the 11 – 13-day-old embryo, the prolongation by E-4031 was prominent and was accompanied by early after-depolarizations (EADs); phase 3 repolarization was interrupted followed by a single or a series of partial depolarization toward 0-mV level (Fig. 1Ac). Early after-depolarizations were observed in all of the preparations from the 11 – 13-day-old embryo (n = 6), but was not observed in those from other stages.

Chromanol 293B (30 μM), a blocker of the slowly activating component of the delayed rectifier K^+ current (I_{Ks}), prolonged action potential duration at all developmental stages (Fig. 1Cb); the prolongation by chromanol 293B was statistically significant, but was much smaller than that by E-4031 at all developmental stages.

Terfenadine (30 μM) produced a prolongation of the action potential duration in the 11 – 13-day-old embryo. Such prolongation was not observed at other stages (Fig. 1B, Cc).

The action potential duration of the chick ventricular myocardium increased during the embryonic period and was shortened in the hatched chick. This was in agreement with previous reports (1, 8) and also appeared to be similar to the case in the guinea pig where the action potential duration increased during the embryonic period followed by a decrease during the perinatal period (9). The developmental change in action potential duration is likely to be accompanied by changes in the balance of underlying membrane currents, as was shown to be the case in guinea pig (10). Such changes would greatly affect the responsiveness of the myocardium to pharmacological agents acting on myocardial ion channels. E-4031 markedly prolonged the action potential duration at all ages examined, while the prolongation by chromanol 293B was small (Fig. 1C). This suggests that in the chick ventricular myocardium, the rapid component (I_{Kr}), but not the slow component (I_{Ks}), of the delayed rectifier potassium channel is largely responsible for the repolarization, which is the same as in many mammalian species including the guinea pig, rabbit, and human. The prolongation by E-4031 was most prominent in the 11 – 13-day-old embryo (Fig. 1). The most likely explanation is that the prolongation of the repolarization phase of the embryonic myocardia reflects less density of repolarizing currents. In other words, the embryonic myocardia have less "repolarization reserve" (11), which makes the action potential highly sensitive to I_{Kr} blockade. Early afterdepolarizations, which are considered to be one of the causes of cardiac arrhythmia, were induced by E-4031 in all of the preparations from the 11 – 13-day-old embryo (Fig. 1Ac). In mammalian myocardia, I_{Kr} blockade is known to induce action potential prolongation, but does not always induce early after-depolarization. For example, in guinea-pig ventricular myocardium, E-4031 (1 μM) prolonged the action potential duration by about 70 ms, but did not induce early after-depolarizations (12).

Clinical treatment with certain cardiovascular and non-cardiovascular drugs has been reported to induce QT prolongation and serious ventricular arrhythmia including *torsades de pointes*. Drugs such as terfenadine and cisapride were withdrawn from clinical practice because of their arrhythmogenic risk. Since then, the assessment of the risks incurred with noncardiovascular therapeutic agents for cardiac function has received great attention (13). Terfenadine inhibits the I_{Kr} current in freshly isolated myocardial cells (14) and the hERG channel current expressed in HEK293 cells (15), but does not prolong the

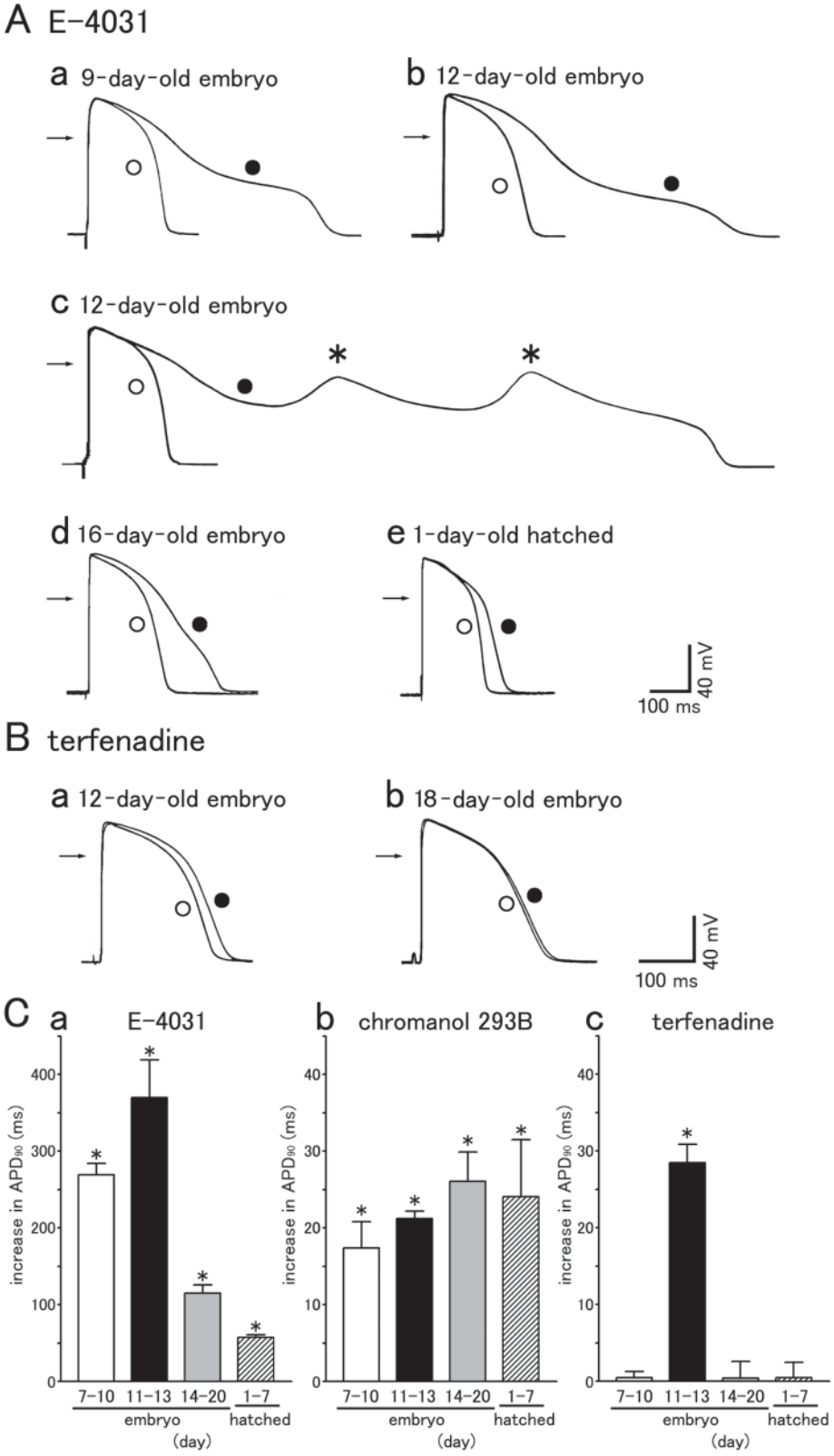


Fig. 1. Effect of K^+-channel blockers on the action potential of isolated chick ventricular myocardium. A: Typical action potential recordings before (open circles) and after (closed circles) the addition of E-4031 (1 μM) in the 9-day-old embryo (a), 12-day-old embryo (b, c), 16-day-old embryo (d), and 1-day-old hatched chick (e). Note that E-4031 induced early after-depolarizations in the 12-day-old embryo (c). B: Typical action potential recordings before (open circles) and after (closed circles) the addition of terfenadine (30 μM) in the 12-day-old embryo (a) and in the 18-day-old embryo (b). Horizontal arrows indicate zero-mV level. Voltage and time calibrations apply to all panels. C: Summarized results for the prolongation of the action potential duration at 90% repolarization (APD_{90}) by 1 μM E-4031 (a), 30 μM chromanol 293B (b), and 30 μM terfenadine (c) in the 7 – 10-day-old embryo (open columns), 11 – 13-day-old embryo (closed columns), 14 – 20-day-old embryo (gray columns), and 1 – 7-day-old hatched chick (hatched columns). Columns and vertical bars are the mean ± S.E.M. from 5 to 6 experiments. Asterisks indicate that the prolongation was statistically significant. Note the difference in the scale of the ordinate.

action potential duration in isolated ventricular tissue from mammals even at 20 μM (15,16). Such presence of "false positive" drugs complicate drug evaluation through action potential duration in tissue preparations (17).

Terfenadine did not prolong action potential duration in the 14 – 20-day-old embryo, which was the same as in isolated mammalian myocardial tissue. However, terfenadine prolonged the action potential duration in the 11 – 13-day-old embryo (Fig. 1: B, Cc). Thus, the 11 – 13-day-old chick embryonic myocardium may be a sensitive model to detect the proarrhythmic activity of drugs. Details such as the density of depolarizing and repolarizing membrane currents and the molecular structure of the delayed rectifier potassium channel in the chick ventricle await further investigation.

In conclusion, we found that the chick ventricle changes its repolarization reserve during development, which provides an interesting model for further studies on myocardial repolarization mechanisms and drug evaluation.

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