



NOTE

Physiology

Partial exposure of frog heart to high-potassium solution: an easily reproducible model mimicking ST segment changes

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ABSTRACT. By simply inducing burn injuries on the bullfrog heart, we previously reported a simple model of abnormal ST segment changes observed in human ischemic heart disease. In the present study, instead of inducing burn injuries, we partially exposed the surface of the frog heart to high-potassium (K⁺) solution to create a concentration gradient of the extracellular K⁺ within the myocardium. Dual recordings of ECG and the cardiac action potential demonstrated significant elevation of the ST segment and the resting membrane potential, indicating its usefulness as a simple model of heart injury. Additionally, from our results, Na⁺/K⁺-ATPase activity was thought to be primarily responsible for generating the K⁺ concentration gradient and inducing the ST segment changes in ECG.

KEY WORDS: bullfrog heart, ischemic heart disease, Na⁺/K⁺-ATPase activity, partial exposure to high-potassium solution, ST segment change

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Ischemic heart disease, such as myocardial infarction and angina pectoris, is among the major causes of morbidity and mortality worldwide [12]. Ischemia induces the large loss of cardiomyocytes, which eventually leads to impaired cardiac contractility, pump dysfunction and the subsequent development of congestive heart failure [14]. Life-threatening ventricular tachyarrhythmia is occasionally provoked by the disease, sometimes leading to sudden cardiac arrest [2]. In our previous study, by simply inducing burn injuries on the bullfrog heart, we reproduced abnormal ST segment changes in the electrocardiogram (ECG), mimicking those observed in ischemic heart disease [7]. Concerning the mechanisms, cellular damage caused by the injuries was thought to make the extracellular concentration of potassium (K⁺) ions higher around the cells [13], causing their resting membrane potential to become significantly higher than that of the adjacent intact cells. In this context, simply creating a K⁺ concentration gradient within the myocardium would affect the ST segment changes in ECG. Therefore, the purpose of our study was to generate an easily reproducible model of myocardial damage in frog hearts and using this model, to reveal the physiological mechanisms of ST segment changes. Here, instead of inducing burn injuries in frog hearts, we partially exposed the surface directly to high-K⁺ solution to obtain ECG abnormalities mimicking those observed in human ischemic heart disease. Then we simultaneously recorded the ECG waves and the action potential of cardiomyocytes. By pharmacological inhibition or stimulation of the cardiac Na⁺/K⁺-ATPase activity, we will also examine the physiological mechanisms underlying the ST segment changes in ECG.

Adult male bullfrogs weighing 400 to 500 g ($n=31$) were purchased from Mr. Ohuchi Kazuo (Ibaraki, Japan). As we previously described [7, 8], they were subjected to intramuscular injection of a long-acting anesthetic, ethyl carbamate (0.50 g/kg; Wako Pure Chemical Industries, Ltd., Osaka, Japan) after initial inhalation with diethyl-ether. Under deep anesthesia, we surgically exposed the frog heart and directly recorded the electrical signals using an ECG amplifier of our own making [7]. We monitored and recorded the ECG waveforms with an oscilloscope (TDS 1002, Tektronix Inc., Beaverton, OR, U.S.A.) and a recorder (Thermal arraycorder Type WR8500, GRAPHTEC Corp., Yokohama, Japan). To obtain the transmembrane action potential, we employed the suction-electrode method. As we described in our previous studies [7, 8], the action potential and the ECG waveforms were recorded simultaneously. All experimental protocols described here were approved by the Ethics Review Committee for Animal Experimentation of Tohoku University.

To partially expose the cardiac muscle to high-K⁺ solution, we gently placed a cotton bar immersed with 1M KCl (Wako Pure

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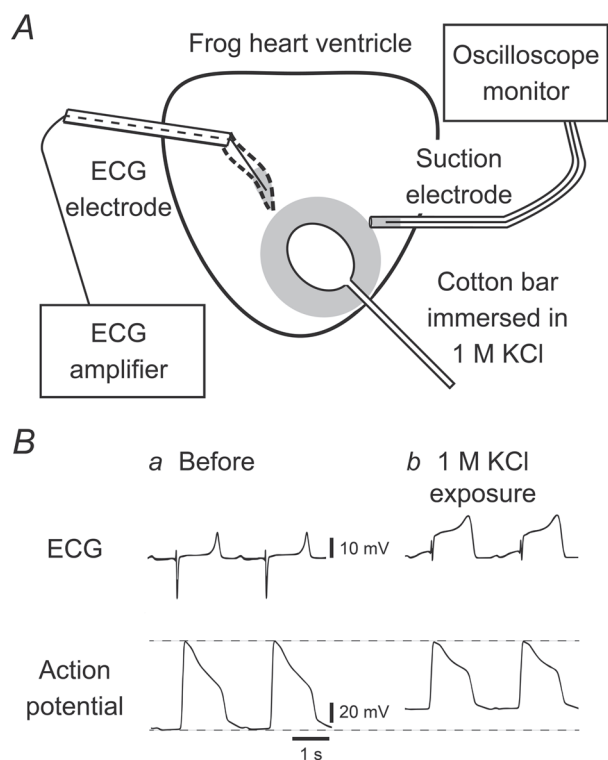


Fig. 1. Partial exposure of high-potassium solution to bullfrog heart and the changes in electrocardiogram (ECG) and the transmembrane action potential. (A) To partially expose the cardiac muscle to high-potassium solution, we gently placed a cotton bar immersed with 1 M KCl solution on the subepicardial myocardium adjacent to the ventricular surface, where the ECG- and the suction- electrodes were placed. (B) The ECG waves (top) and the action potential of ventricular cardiomyocytes (bottom) were simultaneously recorded before (a) and after (b) 1 M KCl exposure. Dashed lines represent the peak of the action potential and the resting membrane potential levels before KCl exposure (baseline levels).

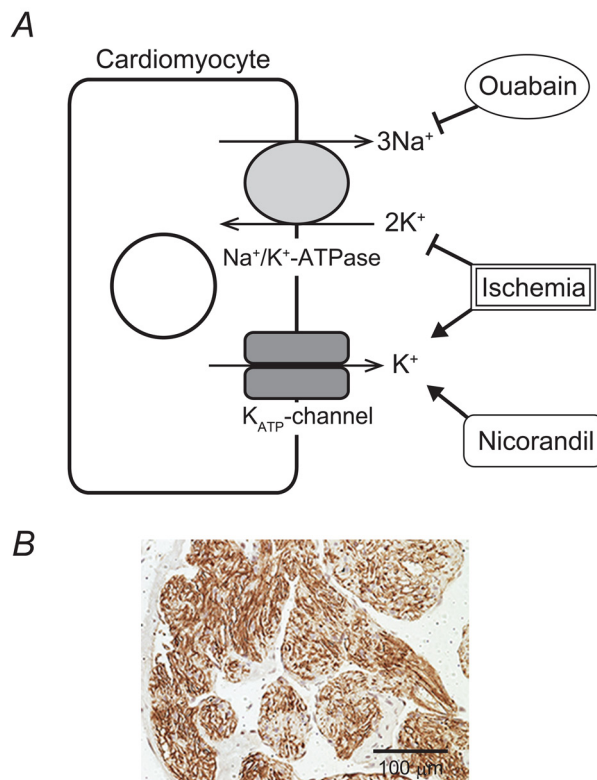


Fig. 2. Ion transport through Na⁺/K⁺-ATPase and K_{ATP}-channels in cardiomyocytes. (A) Na⁺/K⁺-ATPase transports potassium (K⁺) ions into the cell but sodium (Na⁺) ions out of the cell. K_{ATP}-channel facilitates the outward leakage of K⁺ ions. In ischemic conditions, hypoxia diminishes the activity of Na⁺/K⁺-ATPase, but stimulates the activity of K_{ATP}-channels. Exogenously, ouabain inhibits the activity of Na⁺/K⁺-ATPase, while nicorandil stimulates the opening of K_{ATP}-channel. (B) Immunohistochemistry using an antibody for Na⁺/K⁺-ATPase α-1 subunit (brown) in ventricular cardiomyocytes of bullfrog heart, counterstained with hematoxylin. Magnification × 20.

Chemical, Japan) solution several times on the subepicardial myocardium adjacent to the ventricular surface, where the ECG- and the suction- electrodes were placed (Fig. 1A). Before exposing the frog heart to KCl, ECG showed normal QRS complexes followed by positive T waves (Fig. 1Ba top), between which were the ST and TQ segments recorded on the isoelectric line. The simultaneous recording of the action potential demonstrated the excitation and de-excitation of cardiomyocytes (Fig. 1Ba bottom), followed by the resting membrane potential (phase 4) in-between [7, 8]. However, immediately after the 1M KCl exposure, the ECG showed a marked elevation of the ST segment (Fig. 1Bb top, 18.7 ± 1.6 mV increase from the isoelectric line, $n=20$), and the cardiac action potential demonstrated a significant increase in the resting membrane potential (Fig. 1Bb bottom, 18.2 ± 2.0 mV increase from the baseline, $n=20$).

As we have recently shown in a frog heart model with subepicardial burn injuries [7], the partial exposure of high-K⁺ solution to the surface of frog heart similarly reproduced abnormal ST segment changes mimicking those observed in ischemic heart disease [18] (Fig. 1Bb top). Additionally, this frog heart model actually induced a significant elevation of the resting membrane potential in the affected myocardium (Fig. 1Bb bottom). Concerning the mechanisms of the K⁺-induced ST segment elevation, the voltage gradient of the resting membrane potential between the myocardium with high and normal K⁺ concentrations initially generated the “currents of injury” during the diastolic phase [9]. These currents negatively deflected the ECG vector during the diastolic phase and made the ST segment appear elevated during the systolic phase. Similarly to inducing subepicardial burn injuries [7], partially exposing the frog heart to high-K⁺ solution is a simple and easily reproducible procedure. Therefore, this frog heart model would also be suitable for explaining the mechanisms of the ST segment changes observed in human ischemic heart disease.

The resting membrane potential of cardiomyocytes is primarily maintained by the activity of sodium-potassium pump (Na⁺/K⁺-ATPase), which normally transports K⁺ ions into the cell but sodium (Na⁺) ions out of the cell [4] (Fig. 2A). In the frog heart, as previously demonstrated in mammalian hearts [19], the expression of Na⁺/K⁺-ATPase α-1 subunit (1:50; Santa Cruz Biotechnology,

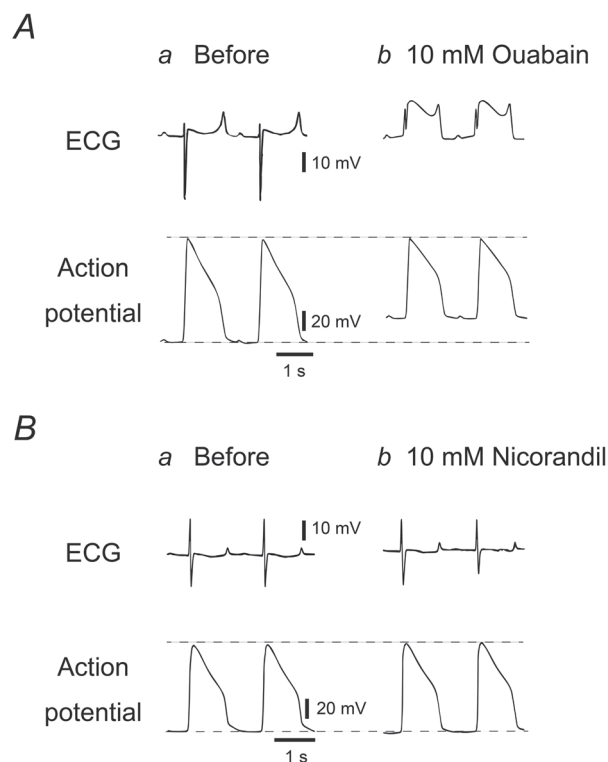


Fig. 3. Effects of ouabain and nicorandil on ECG and the transmembrane action potential. Ventricular surface of frog hearts was partially exposed to 10 mM ouabain (A) or 10 mM nicorandil (B). The ECG waves (top) and the action potential of cardiomyocytes (bottom) were simultaneously recorded before (a) and after (b) the drug exposure. Dashed lines represent the peak of the action potential and the resting membrane potential levels before the drug exposure (baseline levels).

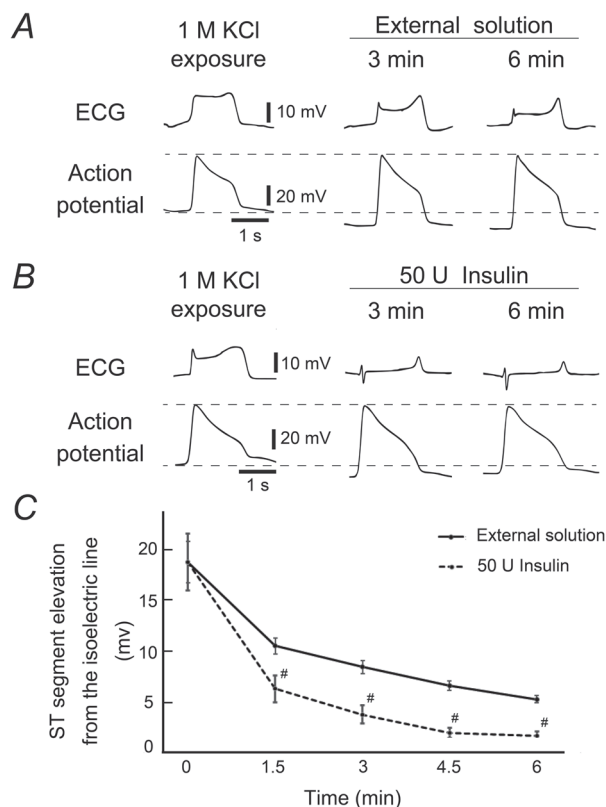


Fig. 4. Effects of insulin on high-potassium-induced changes in ECG and the transmembrane action potential. After partial exposure to 1M KCl, frog hearts were washed out by external solution alone (A) or the external solution containing 50 U insulin (B). The ECG waves (top) and the action potential of cardiomyocytes (bottom) were simultaneously after 1 M KCl exposure, 3 and 6 min after the washout. Dashed lines represent the peak of the action potential and the resting membrane potential levels after KCl exposure. (C) Numerical changes in the ST segment elevation in the frog hearts washed out by the external solution alone and those by the insulin-containing external solution. ST segment elevation was measured after 0, 1.5, 3, 4.5 and 6 min after the washout. # $P < 0.05$ vs. external solution alone. Values are means \pm SEM (external solution alone, $n=11$; insulin-containing external solution, $n=9$). Differences were analyzed by ANOVA followed by Dunnett's or Student's t test.

Inc., Dallas, TX, U.S.A.) was predominantly localized to the plasma membrane throughout the ventricular cardiomyocytes (Fig. 2B). Additionally, the resting membrane potential is also determined by the leakage of K^+ ions through the inwardly rectifying K^+ -channels, such as Kir2.1, Kir3.1 and Kir 6.2 (a major subunit of ATP-sensitive K^+ -channel; K_{ATP}) [6]. In ischemic conditions, including acute myocardial infarction and angina pectoris, cardiac hypoxia decreases the intracellular concentration of adenosine triphosphate (ATP), which diminishes the activity of Na^+/K^+ -ATPase [4], but stimulates the activity of K_{ATP} -channels [11] (Fig. 2A). In the present study, to determine the contribution of Na^+/K^+ -ATPase and K_{ATP} -channels to the elevation of the ST segment and resting membrane potential, we examined the effects of a pump inhibitor, ouabain [3] (Wako Pure Chemical, Japan), and K_{ATP} -channel opener, nicorandil [10] (Tokyo Chemical Industry Co., Ltd., Tokyo, Japan) (Fig. 2A), on the dual recordings of ECG and the cardiac action potentials (Fig. 3). Similar to the findings obtained from the high- K^+ exposure (Fig. 1), the partial exposure of the frog heart to 10 mM ouabain induced a marked elevation of the ST segment in ECG (Fig. 3Ab top vs. 3Aa top, 15.5 ± 1.4 mV increase from the isoelectric line, $n=5$) and a significant increase in the resting membrane potential (Fig. 3Ab bottom vs. 3Aa bottom, 20.8 ± 2.7 mV increase from the baseline, $n=5$). However, 10 mM nicorandil did not significantly affect the levels of the ST segment (Fig. 3Bb top vs. 3Ba top, 0.22 ± 0.22 mV increase from the isoelectric line, $n=6$) or resting membrane potential (Fig. 3Bb bottom vs. 3Ba bottom, 1.67 ± 0.61 mV increase from the baseline, $n=6$). From these results, the

decreased activity of Na⁺/K⁺-ATPase, which prevents K⁺ ions from being pumped back into the cells and thus generates the K⁺ concentration gradient [4] (Fig. 2A), was thought to be primarily responsible for the increase in the cardiac resting membrane potential and the subsequent elevation of the ST segment. On the other hand, as Saito *et al.* previously demonstrated using Kir6.2-null mice [16], K_{ATP}-channels, which facilitate the outward leakage of K⁺ ions [11] (Fig. 2), were not likely to contribute to the changes in the resting membrane potential. As we demonstrated in the present study, Na⁺/K⁺-ATPase was highly expressed throughout the ventricular cardiomyocytes under a physiological condition (Fig. 2B). On the other hand, the expression of K_{ATP}-channels is usually stimulated under ischemic condition [1]. Such difference may be responsible for their differential contribution to the K⁺ concentration gradient. Instead of generating the K⁺ gradient, the opening of K_{ATP}-channels plays a role in conserving scarce energy resources by preventing cellular Ca²⁺ overload and depressing the force development during the cardiac muscle contraction [5]. Additionally, recent studies also revealed that the opening of the channels is deeply associated with the cardiac rhythm regulation by accelerating the repolarization during the phase 3 of the action potential [20, 21].

Finally, we examined the direct effects of Na⁺/K⁺-ATPase activity on the high-K⁺-induced ECG and action potential abnormalities (Fig. 4). After partial exposure to 1M KCl (Fig. 1), the frog hearts were washed out by immersing them in external solution containing (in mM): NaCl, 115; KCl, 2; CaCl₂, 2; MgCl₂, 1; Hepes, 5.0 and Na-Hepes, 5.0 (pH 7.4 adjusted with NaOH) (Fig. 4A). The increased ST segment was gradually restored towards the baseline levels (Fig. 4A top), although it remained significantly high at 6 min after the washout (5.21 ± 0.33 mV above the isoelectric line, $n=11$). However, when frog hearts were washed out by external solution containing 50 units (U) insulin (Nacalai Tesque Inc., Kyoto, Japan), a powerful stimulator of Na⁺/K⁺-ATPase activity [15] (Fig. 4B), the elevated ST segment was restored more quickly (Fig. 4B top), almost reaching the isoelectric line at 6 min after the washout (1.63 ± 0.43 mV above the isoelectric line, $n=9$). In both conditions, the resting membrane potential also tended to become restored significantly towards the baseline levels as early as 3 min after the washout (external solution alone: from 17.5 ± 2.6 to 9.00 ± 2.0 mV above the baseline, $n=11$, $P<0.05$, Fig. 4A bottom; insulin-containing external solution: from 19.1 ± 3.4 to 8.67 ± 1.5 mV above the baseline, $n=9$, $P<0.05$, Fig. 4B bottom). Figure 4C shows the numerical changes in the ST segment elevation in the frog hearts washed out by the external solution alone and those by the insulin-containing external solution. Significant differences were observed at each time point after the washout. These results strongly suggested that the increased activity of Na⁺/K⁺-ATPase, which pumps back K⁺ ions into cardiomyocytes [4] (Fig. 2A), was actually responsible for diminishing the transmembrane K⁺ concentration gradient created by the partial exposure to high-K⁺ solution. Additionally, the results may provide some molecular evidence for the recent clinical findings that early intravenous administration of glucose-insulin-potassium actually improved the prognosis of ST elevation myocardial infarction [17].

In conclusion, by partially exposing the bullfrog heart to high-K⁺ solution, we introduced an easily reproducible model of heart injury mimicking ST segment changes in ECG. The Na⁺/K⁺-ATPase activity was thought to be primarily responsible for generating the K⁺ concentration gradient and inducing the ST segment changes.

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