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NOTE

Physiology

Insulin accelerates recovery from QRS complex widening in a frog heart model of hyperkalemia

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ABSTRACT. Hyperkalemia is one of the most common electrolyte disorders. By injecting various concentrations of potassium chloride (KCl) solutions intravenously into bullfrogs, we demonstrated characteristic electrocardiogram (ECG) abnormalities of hyperkalemia in frog hearts. The widened QRS complexes induced by 100 mM KCl injection were accompanied by an increase in the resting membrane potential in cardiomyocytes and a decreased slope of phase 0 in the action potential. Recording both ECG waveforms and the cardiac action potential enabled us to reveal the mechanisms of hyperkalemia-induced ECG abnormalities. Additionally, pre-treatment with insulin, a powerful stimulator of Na^+/K^+-ATPase activity, significantly accelerated the recovery from the widened QRS complexes in the ECG, demonstrating a pronounced shift of extracellular potassium ions into the intracellular space.

KEY WORDS: bullfrog heart, hyperkalemia, insulin, Na^+/K^+-ATPase activity, widening of QRS complexes

Hyperkalemia is one of the most common electrolyte disorders, usually caused by an excessive intake or ineffective elimination of potassium (K^+) ions, or their excessive release from skeletal muscles [17]. In addition to diseases, such as acute or chronic renal insufficiency, hypoaldosteronism, and rhabdomyolysis, medications that impair urinary K^+ excretion also cause hyperkalemia [8]. In hyperkalemia, patients are often asymptomatic or only present with non-specific symptoms, including nausea, generalized fatigue, muscle weakness, or numbness [17]. However, regardless of the presence or absence of these symptoms, electrocardiogram (ECG) abnormalities, such as a peak in the T waves, prolongation of PR intervals, and the widening of QRS complexes, are often observed [3, 8]. As hyperkalemia progresses, the widened QRS complexes evolve into a sinusoidal shape [19], eventually causing fatal cardiac complications, including ventricular arrhythmias and cardiac arrest [3, 8]. Using cardiomyocytes isolated from rabbit or canine hearts, previous studies revealed the morphological changes in the action potential induced by hyperkalemia [21, 22]. However, correlations with ECG abnormalities have not thoroughly been examined at high serum K^+ concentrations due to the technical difficulties in recording them at the same time. In our previous studies, by simply inducing burn injuries on bullfrog hearts or exposing them to a high-magnesium solution, we reproduced ECG abnormalities that mimicked those observed in acute myocardial infarction or hypermagnesemia [10, 13, 16]. Additionally, by recording the cardiac action potential simultaneously, we revealed the physiological mechanisms underlying such ECG abnormalities [10, 16]. Here, by injecting potassium chloride (KCl) solutions intravenously into bullfrogs, we reproduced typical ECG abnormalities of hyperkalemia in frog hearts. The action potential of cardiomyocytes was simultaneously recorded to reveal the mechanisms of hyperkalemia-induced ECG abnormalities. Additionally, by pre-treating frogs with insulin, we demonstrated the involvement of Na^+/K^+-ATPase activity in the recovery from such ECG abnormalities.

Adult male bullfrogs that weighed 430 to 530 g (n=17) were bought from Ohuchi Shōten (Saitama, Japan). As previously described [10–13, 16], frogs were initially surrendered to inhalation of isoflurane (Pfizer Inc., New York, NY, USA) and intramuscular injection of a long-acting anesthetic, ethyl carbamate (0.50 g/kg; Wako Pure Chemical Industries, Ltd., Osaka, Japan). Under deep sedation, frog hearts were surgically exposed and electrical signals were detected using an ECG electrode linked with an amplifier (Fig. 1) [10–13, 16]. Signals were detected as ECG waveforms and recorded using a data logger (midi LOGGER HV GL2000, GRAPHTEC Corp., Yokohama, Japan) [10, 13]. To monitor the transmembrane action potential of cardiomyocytes, we employed the suction-electrode method, which has been described in our previous studies (Fig. 1) [10–12, 16]. This method enabled the simultaneous recording of ECG waveforms and the cardiac action potential. All experimental

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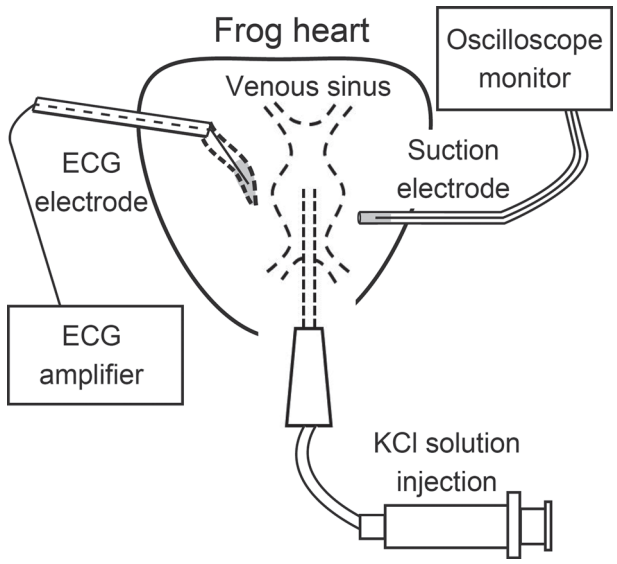

Fig. 1. Intravenous injection of potassium chloride (KCl) solutions and the simultaneous recording of electrocardiogram (ECG) waveforms and the transmembrane action potential. To induce hyperkalemia, 1 ml KCl solutions (1, 10, 100 mM, and 1 M) were separately injected into the venous sinus located on the back of frog hearts. Immediately after each injection, ECG waveforms and the action potential of ventricular cardiomyocytes were simultaneously recorded, using ECG- and suction- electrodes.

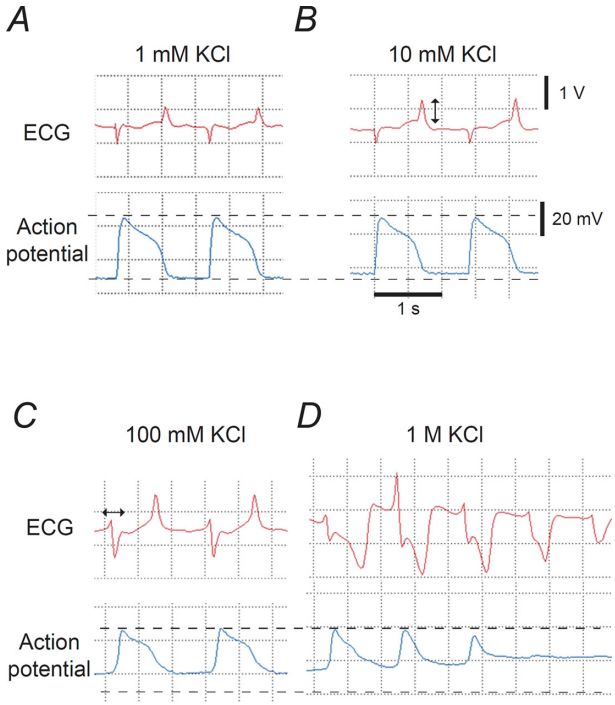

Fig. 2. Effects of potassium chloride (KCl) on electrocardiogram (ECG) and the transmembrane action potential. Bullfrogs were intravenously injected with 1 mM KCl **(A)**, 10 mM KCl **(B)**, 100 mM KCl **(C)**, and 1 M KCl **(D)**. The ECG waveforms (*top*) and the action potential of cardiomyocytes (*bottom*) were simultaneously recorded immediately after each injection.

protocols were approved by the Ethics Review Committee for Animal Experimentation of Miyagi University. Experimental data were analyzed by Microsoft Excel (Microsoft Corp., Redmond, Washington, D.C., USA) and reported as means ± SEM. Statistical significances were assessed by two-way ANOVA followed by Dunnett's or Student's *t* test. A value of $P<0.05$ was considered significant.

To induce hyperkalemia in bullfrogs, different concentrations (1, 10, 100 mM and 1 M) of 1 ml KCl (Wako Pure Chemical Industries) solutions were separately injected into the venous sinus, located on the back of the frog heart (Fig. 1). Then, immediately after each injection, ECG waveforms and the action potential of ventricular cardiomyocytes were simultaneously recorded (Fig. 2). The injection of 1 mM KCl did not affect the ECG waveforms obtained before the injection, showing normal QRS complexes and the following positive T waves (Fig. 2A top). The cardiac action potential was not affected either (Fig. 2A bottom), demonstrating "rapid depolarization (phase 0)", "slow repolarization (combined phase 1 and 2, consisting of partial repolarization and immediately following plateau phase)", "rapid repolarization (phase 3)" and "resting membrane potential (phase 4)" [10–12, 16]. However, after injecting 10 mM KCl (Fig. 2B), the voltage of the T waves tended to increase (811 ± 208 mV vs. 367 ± 92.2 mV before KCl injection; $n=5$; Fig. 2B top), showing peaked T waves (up down arrow). The injection of 100 mM KCl further enhanced peaked T waves (Fig. 2C top). Additionally, a marked increase in the duration of the QRS complexes was shown (229 ± 32.8 msec vs. 115 ± 9.45 msec before KCl injection; $n=7$, $P<0.05$; Fig. 2C top), demonstrating the widening of the QRS complexes (left right arrow). In the simultaneous recording of the cardiac action potential (Fig. 2C bottom), the widened QRS complexes synchronized with a decreased slope of "rapid depolarization (phase 0)" and a marked shift in the resting membrane potential to the depolarized side (Fig. 2C bottom). Finally, injection with an extremely high concentration (1 M) KCl immediately induced ventricular arrhythmia (Fig. 2D), which lasted only a few seconds, eventually causing fatal cardiac arrest.

Hyperkalemia is induced by excess K^+ in the blood above 5.5 mmol/l in humans [8]. In the present study, by intravenously injecting various concentrations of KCl solutions into bullfrogs, we reproduced typical ECG abnormalities of hyperkalemia in frog hearts, such as peaked T waves and the widening of QRS complexes, representing those observed in humans [3, 8]. Concerning the mechanisms of these ECG abnormalities, intravenous KCl administration abruptly elevated the extracellular concentration of K^+ ions, which increased the ratio of the extracellular over the intracellular K^+ concentration [2]. According to the Nernst equation [1], this causes an elevation of the resting membrane potential of ventricular cardiomyocytes to the depolarized side, as we demonstrated in Fig. 2C (bottom). In the cardiac action potential, rapid depolarization during phase 0 is primarily attributable to significant influx of sodium (Na^+) ions through the opening of voltage-gated sodium channels (Nav1.5) [8]. However, in hyperkalemic conditions, the depolarized resting membrane potential causes the steady-state inactivation of the Nav1.5 channels [6,

14]. This slows the rate of rapid depolarization in cardiomyocytes and decreases the slope of phase 0 in the action potential (Fig. 2C bottom). Consequently, such morphological changes in the cardiac action potential manifested as the widening of QRS complexes in the ECG (Fig. 2C top).

In humans, hyperkalemia is usually progressive without prompt treatment to reduce serum K^+ levels, such as the use of insulin with dextrose, salbutamol or sodium bicarbonate [4]. In the present study, to examine the effect of insulin on hyperkalemia-induced ECG abnormalities, we pre-treated bullfrogs with insulin before KCl administration (Fig. 3). Initially, we injected an external solution alone (115 mM NaCl, 2 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 5 mM Hepes and 5 mM Na-Hepes; pH 7.4 adjusted with NaOH) or an external solution containing 10 units (U) insulin (Nacalai Tesque Inc., Kyoto, Japan) into the venous sinus of frog hearts. Then, ECG waveforms and the action potential of cardiomyocytes were simultaneously recorded 30 sec and 2.5 min after 100 mM KCl injection (Fig. 3). Similar to the ECG findings obtained from Fig. 2C, pre-treatment with an external solution alone caused peaked T waves and the widening of QRS complexes 30 sec after KCl injection (Fig. 3A top middle, left right arrow). These changes were correlated with elevated of the resting membrane potential in the cardiac action potential (Fig. 3A bottom middle). After 2.5 min of KCl injection, T waves almost regressed to their normal shape (Fig. 3A top right), but the widened QRS complexes remained unaltered (Fig. 3A top right, left right arrow). Pre-treatment with the insulin-containing external solution also widened the QRS complexes 30 sec after 100 mM KCl injection (Fig. 3B top middle, left right arrow). However, 2.5 min after the KCl injection, the widened QRS complexes regressed to their normal shape (Fig. 3B top right), being almost identical to those prior to the KCl injection (Fig. 3B top left).

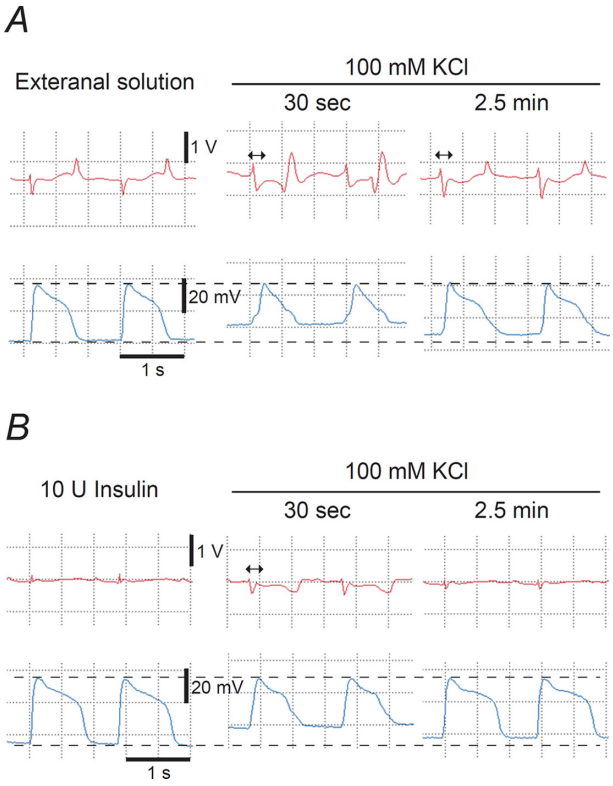

Fig. 3. Effects of insulin on hyperkalemia-induced changes in electrocardiogram (ECG) and the transmembrane action potential. Bullfrogs were initially pre-treated with an external solution alone **(A)** or an external solution containing 10 units (U) insulin **(B)**. Then the ECG waveforms and the action potential of cardiomyocytes were simultaneously recorded 30 sec and 2.5 min after 100 mM potassium chloride (KCl) injection.

From these results, insulin seemingly affects the duration of QRS complexes (Fig. 3B vs. 3A). To clarify this, numerical changes in QRS duration were continuously monitored in frog hearts pre-treated with an external solution alone and those pre-treated with an insulin-containing external solution and compared for 4.5 min after inducing hyperkalemia (Fig. 4A). Regardless of insulin administration, the QRS duration markedly increased 30 sec after 100 mM KCl injection (Fig. 4A). In frog hearts pre-treated with an external solution alone, the increased QRS duration gradually regressed to its baseline level over the 4.5 min observation period (Fig. 4A, solid line with circular makers). However, in frog hearts pre-treated with an insulin-containing external solution, the increased QRS duration regressed more quickly, almost reaching its baseline level even 2.5 min after KCl injection (Fig. 4A, solid line with square markers). During the observation period from 1.5 min to 4 min after KCl injection, significant differences in QRS duration were noted between frog hearts pre-treated with an external solution alone and those pre-treated with an insulin-containing external solution (Fig. 4A). These results clearly demonstrate that pre-treatment with insulin significantly accelerates the recovery from hyperkalemia-induced ECG abnormalities in frog hearts.

In physiological conditions, K^+ ions across the body are predominantly distributed in the intracellular space than in the extracellular space, with skeletal muscles and the liver being the largest reservoirs of K^+ [7] (Fig. 4B). Despite its small occupancy in the total body fluid, extracellular K^+ concentration is usually tightly regulated within a narrow range [2]. This K^+ balance is defined as "potassium homeostasis [7]". Potassium homeostasis is primarily controlled by the ubiquitous sodium-potassium pump (Na^+/K^+-ATPase), which normally transports K^+ ions into the cells, while transporting Na^+ ions out of the cells [2, 20] (Fig. 4B). In the present study, intravenous KCl injection immediately increased extracellular K^+ concentration and induced typical ECG abnormalities associated with hyperkalemia (Figs. 2 and 3A). However, pre-treatment with insulin, a powerful stimulator of Na^+/K^+-ATPase activity [2], facilitated a significant shift of extracellular K^+ ions into the intracellular space (Fig. 4B). This quickly ameliorated the increase in extracellular K^+ concentration and thus accelerated recovery from hyperkalemia-induced ECG abnormalities (Figs. 3B and 4A). Besides insulin, catecholamines are also known to stimulate Na^+/K^+-ATPase activity [2]. Additionally, in several *in vitro* studies, hormones, such as triiodothyronine, aldosterone, and glucagon, functionally stimulated Na^+/K^+-ATPase activity or enhanced its protein expression by increasing mRNA abundance [5, 9, 15, 18, 20]. Concerning the physiological properties of these hormones, they may also be useful in the acute or chronic management of hyperkalemia.

In conclusion, by injecting KCl solutions intravenously into bullfrogs, we reproduced typical ECG abnormalities of hyperkalemia in frog hearts. Simultaneous recordings of the cardiac action potential enabled us to reveal the mechanisms of

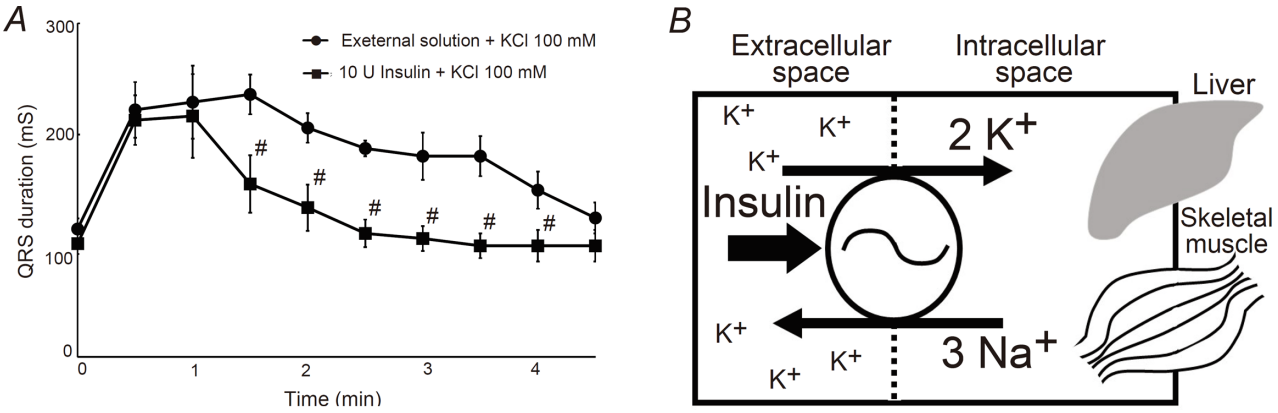

Fig. 4. Effects of insulin on hyperkalemia-induced electrocardiogram (ECG) abnormalities and the mechanisms. **(A)** Bullfrogs were initially pre-treated with an external solution alone or an external solution containing 10 units (U) insulin. Then the numerical changes in the QRS duration in ECG were continuously measured for 4.5 min after 100 mM potassium chloride (KCl) injection. # $P<0.05$ *vs.* external solution alone. Values are means ± SEM (external solution alone, $n=5$; insulin-containing external solution, $n=5$). Differences were analyzed by ANOVA followed by Dunnett's or Student's *t* test. **(B)** Due to potassium (K^+) homeostasis, the total body K^+ ions are more predominantly distributed in the intracellular space than in the extracellular space, with skeletal muscles and the liver being the largest pool of K^+ in the body. Intravenous potassium chloride (KCl) injection immediately increases the extracellular K^+ concentration. However, pre-treatment with insulin, a powerful stimulator of Na^+/K^+-ATPase activity, induces a significant shift of extracellular K^+ into the intracellular space.

hyperkalemia-induced ECG abnormalities. Additionally, pre-treatment with insulin, a powerful stimulator of Na^+/K^+-ATPase activity, significantly accelerated recovery from the widened QRS complexes in ECG, demonstrating a significant shift of extracellular K^+ ions into the intracellular space.

POTENTIAL CONFLICTS OF INTEREST. The authors have nothing to disclose.

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