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NOTE

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High-calcium exposure to frog heart: a simple model representing hypercalcemia-induced ECG abnormalities

Itsuro KAZAMA[1)*]

[1)]Department of Physiology, Tohoku University Graduate School of Medicine, Seiryo-cho, Aoba-ku, Sendai, Miyagi 980-8575, Japan

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ABSTRACT. By simply adding a high concentration of calcium solution to the surface of the bullfrog heart, we reproduced electrocardiogram (ECG) abnormalities representing those observed in hypercalcemia, such as Osborn waves and shortening of the QT interval. The rise in extracellular calcium concentration may have activated the outward potassium currents during phase 3 of the action potential, and thus decreased its duration. In addition to the known decrease in the duration of phase 2, such changes in phase 3 were also likely to contribute to the shortening of the QT interval. The dual recordings of the action potential in cardiomyocytes and the ECG waves enabled us to demonstrate the mechanisms of ECG abnormalities induced by hypercalcemia.

KEY WORDS: action potential, bullfrog heart, hypercalcemia, Osborn waves, shortening of QT interval

Hypercalcemia is caused by a variety of disorders, including primary hyperparathyroidism, malignancy, sarcoidosis, and sometimes, iatrogenic vitamin D intoxication [2]. The clinical manifestations of hypercalcemia are usually mild. However, severe hypercalcemia can cause serious cardiac complications, such as supraventricular or ventricular arrhythmias [13], cardiomyopathy [21] and myocardial calcification, leading to angina pectoris or valvular heart disease [20]. In addition to such cardiac abnormalities, clinical studies have shown that hypercalcemia induces various abnormalities in electrocardiograms (ECG) [5], including prolongation of the PR interval, increased amplitude of the QRS complex, Osborn waves, shortening of the QT interval and elevation of the ST segment [1, 17, 18]. Using cardiac muscles isolated from canine hearts, previous *in vitro* studies revealed morphological changes in the action potential induced by hypercalcemia [24]. However, correlations between ECG abnormalities and changes in the action potential have not been examined precisely in canine or rodent hearts due to technical difficulties in recording them simultaneously. In previous studies, ECGs recorded from bullfrog hearts showed a pattern almost identical to that of humans or rodents [7, 28], indicating its usefulness as a model of the human heart. Recently, by simply inducing burn injuries on the frog heart, we could actually reproduce ECG abnormalities mimicking those observed in ischemic heart disease [12]. Additionally, we could easily record the ECG waves and the action potential of cardiomyocytes simultaneously in the frog heart [12], which enabled us to explain the mechanisms underlying the ECG changes. In this context, the purpose of our present study was to reproduce ECG abnormalities of hypercalcemia in frog hearts to demonstrate their mechanisms. Here, by simply adding a high concentration calcium solution to the surface of the bullfrog heart, we actually reproduced ECG abnormalities representing those observed in hypercalcemia, such as Osborn waves and shortening of the QT interval. In this model, by simultaneously recording the action potential of cardiomyocytes and the ECG waves, we also demonstrated the mechanisms of such ECG changes induced by hypercalcemia.

We purchased adult male bullfrogs, weighing 400–500 g (n=6), from Mr. Ohuchi Kazuo (Ibaraki, Japan) and initially had them inhale diethyl-ether to induce anesthesia. We then administered an intramuscular injection of a long-acting anesthetic, ethyl carbamate (0.50 g/kg; Wako Pure Chemical Industries, Ltd., Osaka, Japan), which was effective during the entire experiment. While deeply anesthetized, the frog heart was surgically exposed, as described in our previous study [12], and the electrical signals were directly recorded. Briefly, we gently placed a silver wire coated with a layer of silver chloride on the surface of the ventricle and connected it to an ECG amplifier made in our laboratory [12]. We monitored the ECG waveforms with an oscilloscope (TDS 1002, Tektronix Inc., Beaverton, OR, U.S.A.), which was connected to a recorder (Thermal arraycorder Type WR310, GRAPHTEC Corp., Yokohama, Japan). As previously described [12], we employed the suction-electrode method for transmembrane potential recording. Briefly, we placed a chloride-coated silver wire, covered with a polyethylene tube 1 mm in

*Correspondence to: Kazama, I., Department of Physiology, Tohoku University Graduate School of Medicine, Seiryo-cho, Aoba-ku, Sendai, Miyagi 980-8575, Japan. e-mail: kazaitsu@med.tohoku.ac.jp

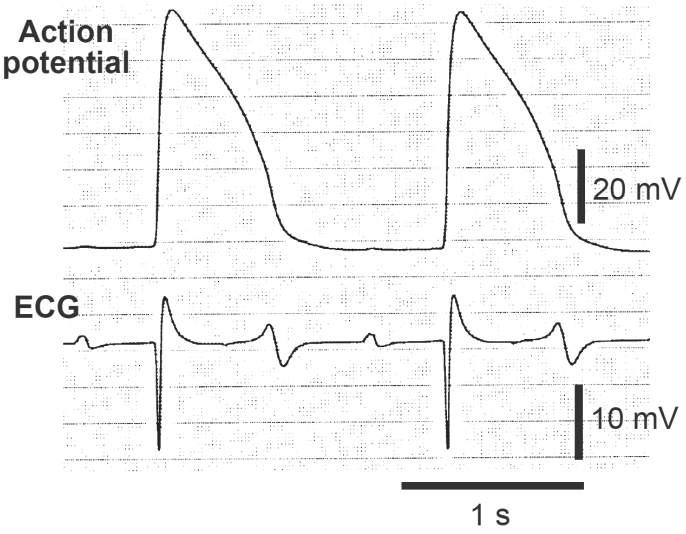

Fig. 1. Simultaneous recordings of the transmembrane action potential and electrocardiogram (ECG) in the bullfrog heart. The top trace shows the action potential of ventricular cardiomyocytes, which consists of phases including "overshoot" (phase 0), "slow repolarization" (combined phase 1 and 2: partial repolarization immediately followed by the plateau phase), "rapid repolarization" (phase 3) and "resting membrane potential" (phase 4). The bottom trace illustrates the ECG with P waves followed by prominent QRS complexes and biphasic T waves.

diameter, on the surface of the ventricle and connected it to the amplifier. We filled the tube with external solution containing (in mM) NaCl (115), KCl (2), $CaCl_2$ (2), $MgCl_2$ (1), Hepes (5) and Na-Hepes (5) at pH 7.4. Using a syringe connected to the tube, we applied negative pressure to the recording electrode to break the cellular membranes in the tube. All experimental protocols described herein were approved by the Ethics Review Committee for Animal Experimentation of Tohoku University.

The simultaneous application of the electrical poles and the suction-electrode technique to the ventricular muscle enabled dual recording of the ECG of the heart and the action potential of cardiomyocytes, as shown in Fig. 1. Consistent with our previous findings [12], the top trace of Fig. 1 illustrates the action potential of the ventricular cardiomyocytes, composed of phases including "overshoot" (phase 0), "slow repolarization" (combined phase 1 and 2: partial repolarization immediately followed by the plateau phase), "rapid repolarization" (phase 3) and "resting membrane potential" (phase 4). The bottom trace shows the ECG with P waves followed by prominent QRS complexes and biphasic T waves, indicating the normal atrial to ventricular conduction of the electrical stimuli [28]. As demonstrated in our previous study [12], the QRS complex and the following T wave of the ECG temporally coincided with the overshoot (phase 0) and the rapid repolarization (phase 3) of the action potential (Fig. 1). The results confirmed our previous findings that the QRS complex and the T wave represent the excitation and de-excitation of cardiomyocytes, respectively [12, 28].

To examine the direct effects of hypercalcemia on the cardiac muscle, we immersed the ventricular surface of the exposed frog heart in 1 M $CaCl_2$ (Wako Pure Chemical) solution. Thus, we induced a high calcium concentration in the extracellular fluid around the heart. Then, shortly (2 min) and long (30 min) after the immersion of the heart in $CaCl_2$, the action potential of ventricular cardiomyocytes and ECG waves were simultaneously recorded as shown in Fig. 2. Two minutes after immersion in $CaCl_2$ (Fig. 2A), the slope of phase 2 of the action potential became steeper, and its duration was shortened (Fig. 2A top vs. Fig. 1 top). However, the duration of the total systolic phase remained unchanged (751 ± 30.2 msec vs. 777 ± 16.7 msec; n=6, P>0.05), indicating that the duration of rapid repolarization (phase 3) had not decreased. The simultaneous ECG recording showed a hump configuration at the end of the widened QRS complexes (Fig. 2A bottom, arrowhead). This indicated the emergence of Osborn (or J) waves, as occasionally reported in patients with severe hypercalcemia [18]. Then, 30 minutes after immersion in $CaCl_2$ (Fig. 2B), the slope of phase 3 of the action potential became steeper (Fig. 2B top vs. Fig. 2A top), and the duration of the total systolic phase became significantly shorter (777 ± 16.7 msec vs. 586 ± 12.9 msec; n=6, P<0.05), indicating a decrease in the duration of phase 3. At this stage, the simultaneous ECG recording demonstrated the absence of Osborn waves (Fig. 2B bottom). The shapes of the QRS complexes and the following T waves were almost identical to those observed prior to immersion in $CaCl_2$ (Fig. 2B bottom vs. Fig. 1 bottom). However, the QT interval, which is defined from the beginning of the QRS complex to the end of the T wave (Fig. 2B bottom, double arrows), was remarkably shortened (807 ± 18.4 msec vs. 557 ± 9.55 msec; n=6, P<0.05), as frequently reported in patients with hypercalcemia [1, 8].

Osborn waves are most commonly observed in patients with hypothermia [19]. These waves have also been observed in other pathological conditions, such as brain injury, subarachnoid hemorrhage, ventricular fibrillation, cardiopulmonary arrest, and sometimes, in severe hypercalcemia [3, 11, 18]. Concerning the mechanisms by which the Osborn waves are formed, previous studies demonstrated an electrophysiological difference between the ventricular epicardium and endocardium in the transient outward potassium current (I_{to}) during phase 1 of the action potential [15]. Using canine hearts, Di Diego *et al.* further revealed that a rise in extracellular calcium actually increased I_{to} in the ventricular epicardium [4]. In hypercalcemia, calcium ions in the

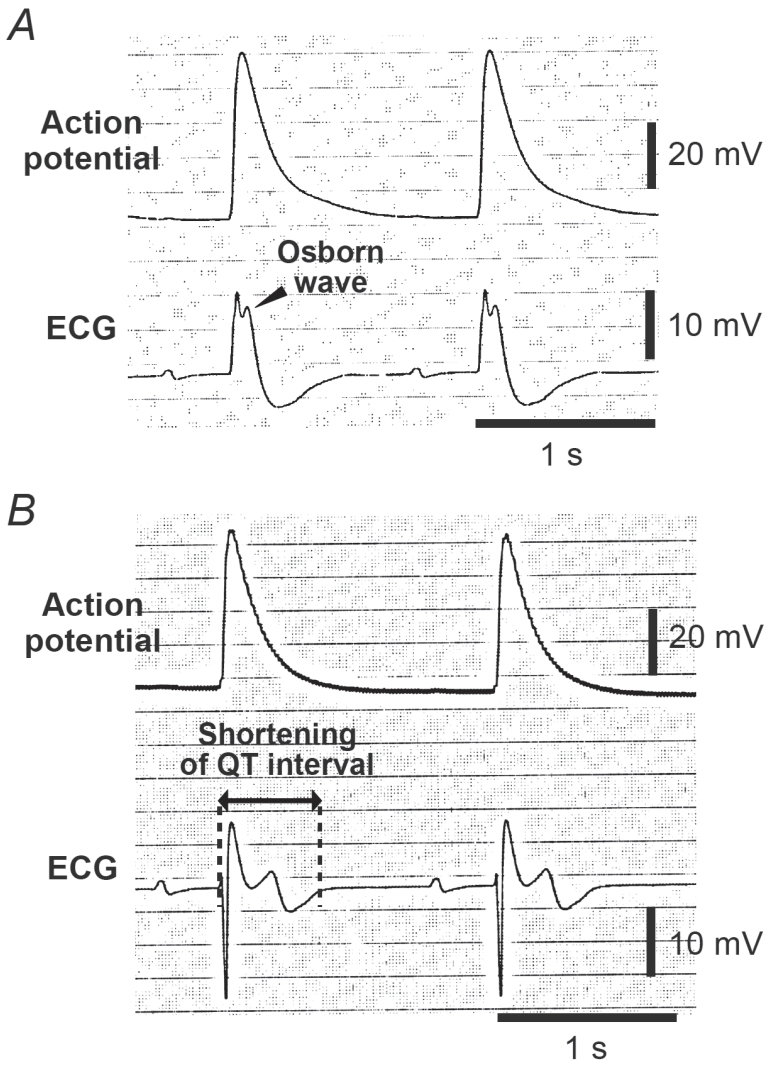

Fig. 2. Effects of high-calcium exposure to the bullfrog heart. $CaCl_2$ solution (1 M) was added externally to immerse the exposed ventricular surface of the frog heart. Shortly (2 min) (A) and long (30 min) (B) after such high-calcium exposure, the action potential of ventricular cardiomyocytes (top) and the ECG waves (bottom) were simultaneously recorded.

blood gradually penetrate into the epicardium from inside the endocardium. Therefore, the epicardium will be affected only when the hypercalcemia is very severe. In the present study, however, since we externally delivered a high concentration of calcium onto the ventricular surface, the calcium ions were thought to directly affect the cardiac muscle from outside the epicardium. This effect easily generated I_{to}-induced electrical heterogeneity between the epicardium and the endocardium, thereby causing the Osborn waves shortly after the addition of $CaCl_2$ (Fig. 2A bottom).

In the present study, as frequently observed in patients with mild to moderate hypercalcemia [1, 8, 23], the ECG recorded from the frog heart demonstrated a prominent shortening of the QT interval 30 min after the external addition of $CaCl_2$ (Fig. 2B bottom). Concerning the mechanisms of such ECG changes, the decreased duration of phase 2 of the action potential, which is determined by the inward calcium flow through L-type calcium channels, is considered primarily responsible [23]. Using a mathematical model of human cardiomyocytes, Grandi *et al.* further demonstrated that a higher calcium concentration in the extracellular fluid would facilitate the rapid influx of calcium through the channels [9]. This enables the intracellular calcium ions to quickly reach the threshold to close the L-type calcium channels, reducing the duration of phase 2 of the action potential. In the present study, however, as we showed in Fig. 2A, the decrease in the duration of phase 2 alone did not change the QT interval. Rather, as previously shown in Purkinje fibers isolated from canine heart [24], shortening of the QT interval occurred only after the duration of phase 3 was also decreased (Fig. 2B). These findings strongly suggested that the decrease in duration of phase 3 was also likely to contribute to the shortening of the QT interval.

Generally, the duration of phase 3 in the action potential is determined by the outward potassium flow through delayed rectifier potassium channels [23] (Fig. 3). However, these channels, including the rapid (IK_r) and slow (IK_s) components of the repolarizing currents, are purely voltage-dependent and are not dependent on the extracellular (or intracellular) calcium concentration [22]. Recently, based on findings in the nervous system [16], two types of calcium-activated potassium channels, the large-conductance

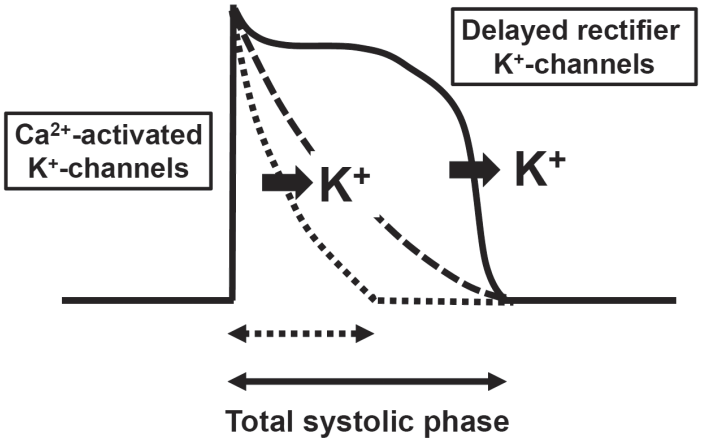

Fig. 3. Mechanisms by which high-calcium exposure decreases the duration of the total systolic phase of the action potential. Action potentials 2 min (dashed line) and 30 min (dotted line) after 1 M $CaCl_2$ was added externally onto the ventricular surface of frog hearts. Generally, the duration of phase 3 (rapid repolarization) of the action potential is determined by the outward potassium flow through the "delayed rectifier potassium channels". However, the rise in extracellular calcium concentration, which facilitates the intracellular entry of calcium ions, stimulates "calcium-activated potassium channels," such as large conductance and small conductance K^+ channels. This would increase the outward potassium currents during phase 3 of the action potential, accelerating the process of repolarization and thus decreasing the total duration of the systolic phase.

and small-conductance K^+ channels (BK and SK, respectively), were additionally identified in human and murine cardiomyocytes [25, 27, 29]. In previous studies, changes in the intracellular calcium concentration of cardiomyocytes actually affected the total length of the action potential [10]. Therefore, in the present study, the rise in extracellular calcium concentration, which facilitates the intracellular entry of calcium ions [9], may have activated these potassium channels in the frog heart. This would increase the outward potassium currents during phase 3 of the action potential (Fig. 3), which accelerates the process of repolarization and thus decreases the total duration of the systolic phase.

In contrast to hypercalcemia, the ECG changes induced by hypocalcemia are frequently characterized by prolongation of the QT interval, which sometimes causes a serious type of ventricular tachycardia called "torsades de pointes" [6, 14]. Since prolongation of the QT interval has been associated with the extension of phase 2 of the action potential [6, 14], intravenous supplementation of calcium is usually primarily required in the management of arrhythmia. However, considering the involvement of calcium-activated potassium channels during phase 3 of the action potential (Fig. 3), which may be deactivated in cases of hypocalcemia, the use of channel activators, such as ethylbenzimidazolone (EBIO), NS1619 and SKA-31, would also be beneficial [26].

In conclusion, using the bullfrog heart, we introduce a simple model representing the ECG abnormalities observed in hypercalcemia, such as Osborn waves and shortening of the QT interval. Dual recording of the action potential in cardiomyocytes and ECG waves enabled us to demonstrate the mechanisms underlying such ECG changes induced by hypercalcemia.

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