The Journal of Veterinary Medical Science

NOTE

Physiology

High-magnesium exposure to bullfrog heart causes ST segment elevation

Itsuro KAZAMA[1)]*

[1)]School of Nursing, Miyagi University, Gakuen, Taiwa-cho, Kurokawa-gun, Miyagi 981-3298, Japan

J. Vet. Med. Sci.
83(4): 675–679, 2021
doi: 10.1292/jvms.20-0720

Received: 21 December 2020
Accepted: 9 February 2021
Advanced Epub:
22 February 2021

ABSTRACT. Hypermagnesemia occurs in elderly people or patients with renal insufficiency after excessive ingestion of magnesium-containing laxatives. In addition to typical electrocardiogram (ECG) findings caused by conduction defects, changes in the ST segments and T waves are also observed in patients with severe hypermagnesemia. This suggested the involvement of similar pathophysiology to acute myocardial infarction, as we previously demonstrated using burn-induced subepicardial injury model in frog hearts. In the present study, by exposing the bullfrog heart to high-magnesium solution, we reproduced prominent ST segment changes in ECG as actually observed in patients with severe hypermagnesemia. In addition to the great increase in the T waves, the ECG showed a marked elevation of the ST segments and the cardiac action potential demonstrated a marked shift of the resting membrane potential to the depolarized side. High-magnesium exposure did not affect the abundance of Na^+/K^+-ATPase proteins. However, the pharmacological stimulation of Na^+/K^+-ATPase activity by insulin quickly retrieved the elevated ST segments in ECG. From these results, the functional blockade of Na^+/K^+-ATPase activity by magnesium ions was thought to be responsible for generating the potassium concentration gradient and the subsequent ST segment changes.

KEY WORDS: acute myocardial infarction, bullfrog heart, Na^+/K^+-ATPase activity, severe hypermagnesemia

Hypermagnesemia is one of the common electrolyte disorders, which rarely occurs in patients with normal renal function. It predominantly occurs in elderly people or patients with renal insufficiency, after they continuously take excessive amounts of magnesium-containing laxatives [21]. Patients with hypermagnesemia are often asymptomatic or only present with nonspecific symptoms, such as nausea, vomiting, flushing and muscle weakness [17]. In mild to moderate hypermagnesemia, electrocardiogram (ECG) changes, including prolongation of the PR- or QT- intervals and the widening of the QRS complexes, are often observed, representing the cardiovascular dysfunction caused by conduction defects [14]. In severe hypermagnesemia, fatal cardiovascular complications, such as cardiac arrest and cardiogenic shock, occasionally occur, for which the progression of the conduction defects is considered primarily responsible [14]. Additionally, in some patients with severe hypermagnesemia, the prominent ST segment elevation and the T wave increase were also observed in ECG [1, 8]. These findings strongly suggested the involvement of similar pathophysiology to acute myocardial infarction in severe hypermagnesemia. In our previous studies, by simply inducing burn injuries on bullfrog hearts or partially exposing their surface to high-potassium (K^+) solution, we reproduced ECG changes of human ischemic heart disease and revealed their physiological mechanisms [9, 11, 13]. Here, by exposing the surface of frog hearts to high magnesium solution, we could reproduce prominent ST segment changes in ECG as actually observed in patients with severe hypermagnesemia [1, 8]. Additionally, by pharmacologically stimulating the cardiac Na^+/K^+-ATPase activity, we revealed the physiological mechanisms underlying such ST segment changes induced by hypermagnesemia.

Adult male bullfrogs weighing 430 to 530 g (n=38) were purchased from Ohuchi Shōten (Saitama, Japan). As we described previously [9–11, 13], the frogs 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 isoflurane (Pfizer Inc., New York, NY, USA). Under deep anesthesia, the frog hearts were surgically exposed and the electrical signals were directly recorded by ECG amplifier [9–11, 13]. The ECG waveforms were monitored and recorded in a data logger (midi LOGGER HV GL2000, GRAPHTEC Corp., Yokohama, Japan) [11]. To detect the transmembrane action potential, the suction-electrode method was employed as we described previously [9, 10, 13]. This enabled the action potential to be simultaneously recorded with the ECG waveforms. All experimental 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 significance was assessed by two-way ANOVA followed by Dunnett's or Student's t test. A value of $P<0.05$ was considered significant.

*Correspondence to: Kazama, I.: kazamai@myu.ac.jp

In patients with severe hypermagnesemia, serum magnesium concentration reached as high as 5 mM, when ECG showed diffuse ST segment elevation and the T wave increase [1, 8]. Therefore, in the present study, to examine the direct effects of severe hypermagnesemia on the cardiac muscle, we externally added as high as 1, 10, 100 mM and 1 M magnesium chloride ($MgCl_2$; Wako Pure Chemical) solution to immerse the ventricular surface of the exposed frog heart. Thus, we induced a high magnesium concentration in the extracellular fluid around the heart. As shown in Figs. 1 and 2, the ECG waveforms and the action potential of ventricular cardiomyocytes were simultaneously recorded continuously for 7 min. Before exposing the frog heart to $MgCl_2$, the ECG showed normal QRS complexes that were followed by positive T waves (Figs. 1A, 1B and 2A, 2B top left). Between the QRS complexes and T waves, there were the ST segments recorded on the isoelectric line. The simultaneous recording of the action potential demonstrated the depolarization and the repolarization of cardiomyocytes (Figs. 1A, 1B and 2A, 2B bottom left), followed by the resting membrane potential between the waves [9, 10, 13]. The exposure of 1 mM $MgCl_2$ to the frog heart did not affect the T waves or ST segments of the ECG and the cardiac action potential (Fig. 1A). However, after the exposure of 10 mM $MgCl_2$ (Fig. 1B), the T waves gradually became upright and showed a slight increase in the voltage (Fig. 1B top). The exposure of 100 mM $MgCl_2$ caused more marked increase in the T waves (Fig. 2A top) and the cardiac action potential demonstrated a significant shift of the resting membrane potential to the depolarized side after 5–7 min (Fig. 2A bottom, 6.7 ± 0.83 mV shift from the baseline, n=7). Shortly (2 min) after the exposure of 1 M $MgCl_2$ (Fig. 2B), in addition to the great increase in the T waves (Fig. 2B top), the ECG showed a marked elevation of the ST segments which became more prominent after 5–7 min (584 ± 156 mV increase from the isoelectric line, n=7). The cardiac action potential also demonstrated a marked shift of the resting membrane potential to the depolarized side which lasted during the observation period (Fig. 2B middle, 8.6 ± 1.5 mV shift after 5–7 min exposure from the baseline, n=9). To detect the action potential of inner cardiomyocytes, a winged needle connected to a polyethylene tube was directly placed into the cardiac muscle and the suction-electrode method was applied as we described

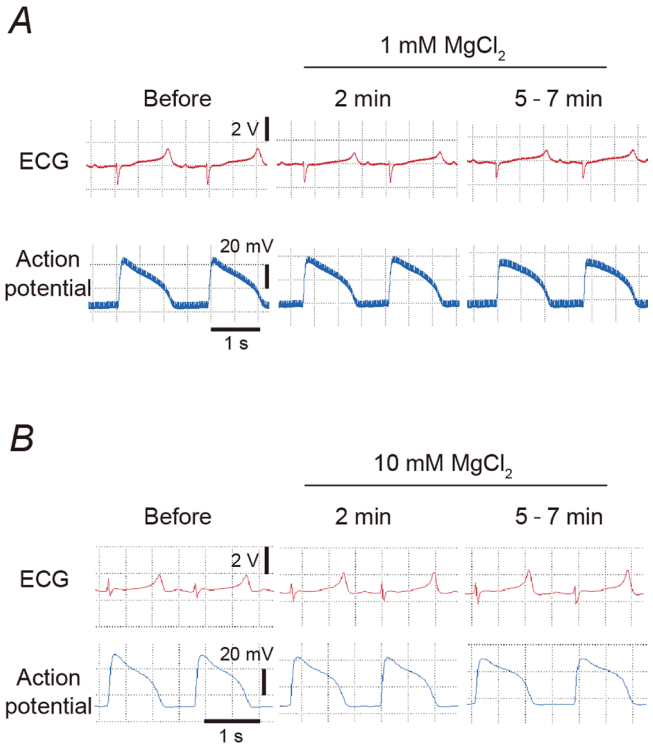

Fig. 1. Effects of 1 mM and 10 mM magnesium chloride ($MgCl_2$) on electrocardiogram and the transmembrane action potential. Ventricular surface of frog hearts was exposed to 1 mM $MgCl_2$ (*A*) or 10 mM $MgCl_2$ (*B*). The electrocardiogram (ECG) waves (*top*) and the action potential of cardiomyocytes (*bottom*) were simultaneously recorded before and 2, 5–7 min after $MgCl_2$ exposure.

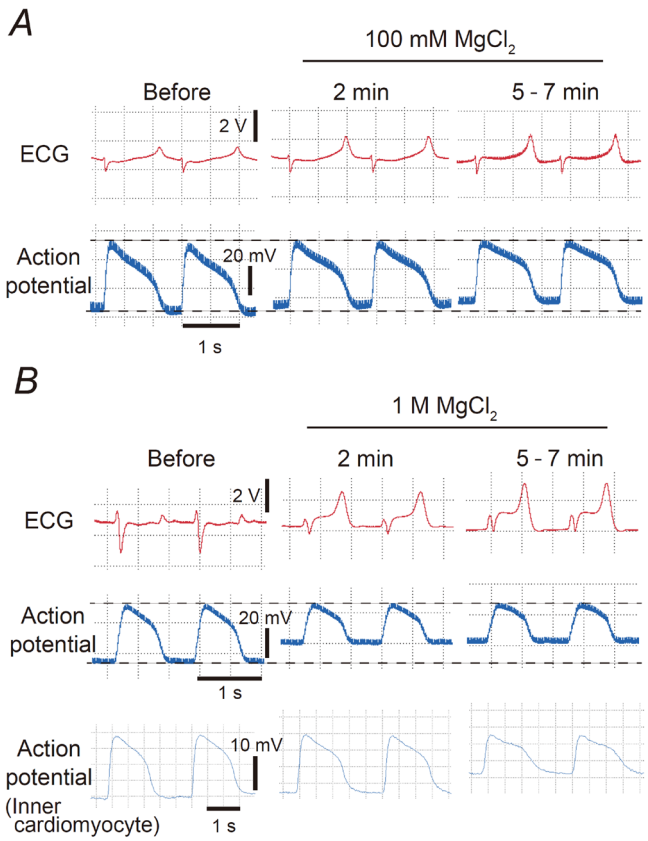

Fig. 2. Effects of 100 mM and 1 M magnesium chloride ($MgCl_2$) on electrocardiogram and the transmembrane action potential. Ventricular surface of frog hearts was exposed to 100 mM $MgCl_2$ (*A*) or 1 M $MgCl_2$ (*B*). The electrocardiogram (ECG) waves (*top*) and the action potential of cardiomyocytes (*middle*) were simultaneously recorded before and 2, 5–7 min after $MgCl_2$ exposure. Dashed lines represent the peak of the action potential and the resting membrane potential levels before the drug exposure (baseline levels). The action potential of inner cardiomyocytes (*bottom*) was also recorded before and 2, 5–7 min after 1 M $MgCl_2$ exposure.

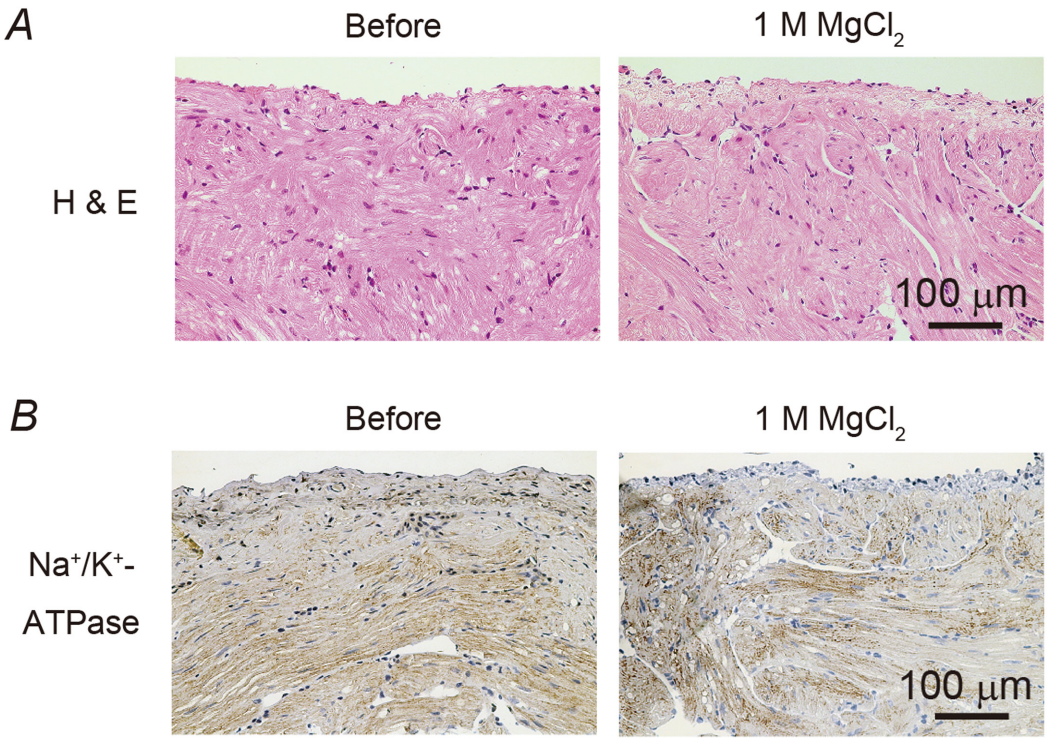

Fig. 3. Morphological changes in ventricular surface and Na^+/K^+-ATPase expression after high-magnesium exposure to bullfrog heart. (*A*) Hematoxylin and eosin (H&E) staining in ventricular cardiomyocytes before (Before) and after 1 M magnesium chloride ($MgCl_2$) exposure (1M $MgCl_2$). Magnification × 20. (*B*) Immunohistochemistry using an antibody for Na^+/K^+-ATPase α-1 subunit (brown), counterstained with hematoxylin in ventricular cardiomyocytes before (Before) and after 1 M $MgCl_2$ exposure (1 M $MgCl_2$). Magnification × 20.

previously [9, 13]. In contrast to the cardiac action potential obtained from the suction-electrode method (Fig. 2B middle), the resting membrane potential of inner cardiomyocytes remained almost unaltered 2 min after the exposure of 1 M $MgCl_2$ (Fig. 2B bottom). However, the resting membrane potential finally shifted to the depolarized side after 5–7 min, indicating that high-magnesium solution may have penetrated deep into the inner cardiac muscle.

Similarly to our frog heart models with subepicardial burn injuries [9] or the partial exposure of high-K^+ solution [13], the exposure of high-magnesium solution reproduced prominent ST segment elevation, mimicking the typical ECG findings observed in human acute myocardial infarction [20] (Fig. 2B top). Additionally, it was accompanied by a significant shift of the resting membrane potential of cardiomyocytes to the depolarized side (Fig. 2B middle). In the present study, since we used extremely high concentrations of magnesium, we could not completely exclude the influence of changes in the osmolarity or pH in the external solution. However, previous studies using frog hearts demonstrated that the changes in the osmolarity or pH in the external solution alone did not significantly affect the resting membrane potential of cardiomyocytes [15, 16]. Regarding the mechanisms that underlie the ST segment elevation, the difference in the resting membrane potential between the high-magnesium exposed- and non-exposed- cardiomyocytes produced the "currents of injury" in the diastolic phase [12]. As we previously demonstrated in our frog heart models with subepicardial burn injuries [9, 11] or the partial exposure of high-K^+ solution [13], the currents deflected the ECG vector negatively during the diastolic phase and made the ST segment look elevated in the systolic phase.

On the surface of the ventricle in normal frog hearts, there were massive layers of cardiac muscles (Fig. 3A, left). The myocardium was covered by the epicardium, which was composed of a layer of mesothelial cells and the fibrous connective tissue below. From our results, the exposure of 1 M $MgCl_2$ induced apparent changes in both ECG waveforms and the cardiac action potential (Fig. 2B). However, such high concentration of $MgCl_2$ did not cause any morphological changes in the surface of the frog heart ventricle and the cardiac muscles below (Fig. 3A, right). Na^+/K^+-ATPase is an ion pump, through which sodium (Na^+) ions are actively transported out of the cell and K^+ ions into the cell [6]. Consistent with our previous results [11, 13], immunohistochemistry for Na^+/K^+-ATPase α-subunit (1:50; Santa Cruz Biotechnology, Inc., Dallas, TX, USA) revealed this protein expression ubiquitously throughout the cellular membrane of cardiomyocytes (Fig. 3B left). In our recent study, the loss of this pump expression in injured cardiomyocytes was deeply associated with the ST segment elevation in ECG, as it causes K^+ concentration gradient across the cellular membrane [11]. In the present study, since the exposure of 1 M $MgCl_2$ showed a similar ST segment elevation in the frog hearts (Fig. 2B top), we examined the changes in Na^+/K^+-ATPase protein expression after the high-magnesium exposure (Fig. 3B, right). However, differing from the findings obtained in our burn-induced subepicardial injury model [11], the protein expression of Na^+/K^+-ATPase was almost totally intact, showing that high-magnesium exposure did not affect the abundance of Na^+/K^+-ATPase proteins.

From these results, the "expression" of Na^+/K^+-ATPase was not likely to be associated with the high-magnesium-induced elevation of

the ST segments. Therefore, we finally examined the direct effects of this pump "activity" on the high-magnesium-induced ECG abnormality (Fig. 4). After exposing 1M $MgCl_2$ to frog hearts for 5 min, they were washed out by immersing them in the external solution composed of 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) (Fig. 4A). The elevated ST segments slowly retrieved towards the baseline level, although they still remained significantly higher than the baseline level at 5–6 min after the washout (from 651 ± 232 mV to 538 ± 166 mV above the isoelectric line, n=5). In contrast, after we washed out the frog hearts by the external solution containing 50 units (U) insulin (Nacalai Tesque Inc., Kyoto, Japan), which powerfully stimulates the Na^+/K^+-ATPase activity [11, 13, 19] (Fig. 4B), the increased ST segments retrieved more rapidly, closely approaching the isoelectric line at 5–6 min after the washout (from 813 ± 231 mV to 234 ± 53.3 mV above the isoelectric line, n=4). Such differences in the ST segment elevation between the frog hearts washed out by the external solution and those by the insulin-containing solution were quantitatively compared during the observation period (Fig. 4C). Significant difference was noticed at 2–3 min after the washout, which became more prominent at 5–6 min.

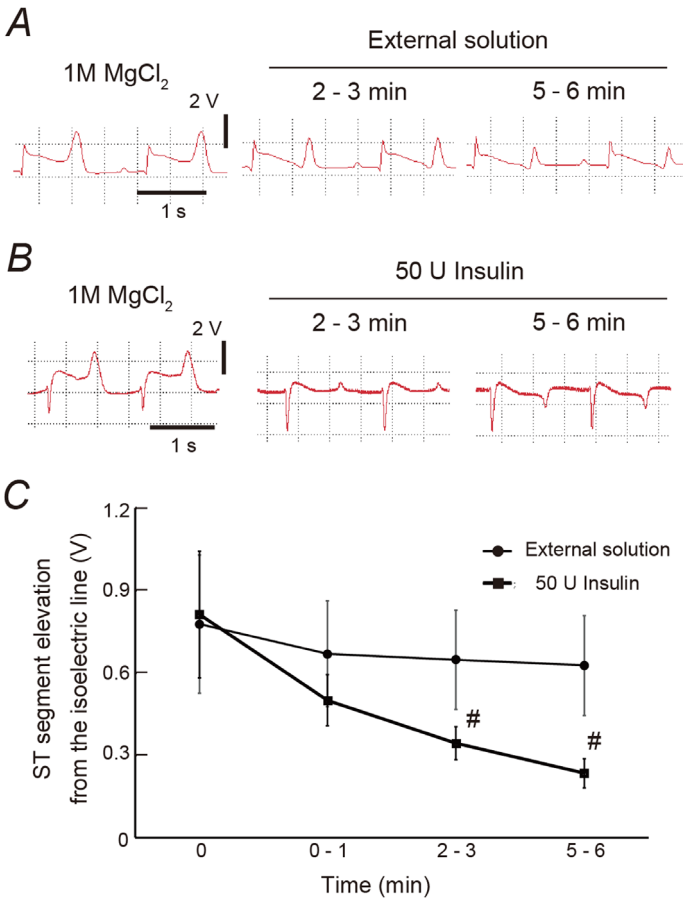

Fig. 4. Effects of insulin on high-magnesium-induced changes in electrocardiogram. After exposing to 1 M magnesium chloride ($MgCl_2$), frog hearts were washed out by external solution alone (*A*) or the external solution containing 50 U insulin (*B*). The electrocardiogram (ECG) waveforms were recorded 5 min after 1 M $MgCl_2$ exposure, and 2–3 and 5–6 min after the washout. (*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 0, 0–1, 2–3 and 5–6 min after the washout. $^{\#}P<0.05$ *vs.* external solution alone. Values are means ± SEM (external solution alone, n=5; insulin-containing external solution, n=4). Differences were analyzed by ANOVA followed by Dunnett's or Student's t test.

In ischemic heart disease, such as acute myocardial infarction and angina pectoris, hypoxia decreases the cytosolic adenosine triphosphate (ATP) concentration in cardiomyocytes. This functionally blocks the activity of Na^+/K^+-ATPase, through which Na^+ and K^+ ions are transported in an ATP-dependent manner [5, 6]. Such functional inhibition of this pump activity was the primary mechanism underlying the ST segment changes observed in ischemic heart disease [13]. In our previous study, to determine the contribution of Na^+/K^+-ATPase to the elevation of the ST segment and resting membrane potential, we actually examined the effects of a pump inhibitor, ouabain on the dual recordings of ECG and the cardiac action potentials [13]. Similar to the findings obtained from the high-magnesium exposure in the present study (Fig. 2B), 10 mM ouabain induced a marked elevation of the ST segment in ECG and a significant shift of the resting membrane potential to the depolarized side [13]. In the present study, despite the absence of "expressional" involvement of Na^+/K^+-ATPase (Fig. 3), its increased activity by insulin, which accelerates the K^+ transport into cardiomyocytes [5], restored the ST segment elevation caused by the high-magnesium exposure (Fig. 4). This indicated the "functional" involvement of Na^+/K^+-ATPase in the magnesium-induced ST segment elevation. At high concentrations, magnesium ions (Mg^{2+}) are known to inhibit the activity of Na^+/K^+-ATPase from inside the cells [3, 18]. Recent advances in molecular biology further revealed that Mg^{2+} ions bind to the pump from the cytoplasmic side and induce conformational changes in the protein structure [2, 7]. Thus, in cardiomyocytes exposed to high-magnesium solution, the inward transportation of K^+ ions was inhibited, which increased their extracellular concentration but decreased their intracellular concentration. According to the Nernst equation [4], this generated a voltage difference in the resting membrane potential between the high-magnesium exposed- and non-exposed- cardiomyocytes, consequently inducing the ST segment elevation [11, 13].

In conclusion, by exposing the bullfrog heart to high-magnesium solution, we reproduced prominent ST segment changes in ECG as actually observed in patients with severe hypermagnesemia. The functional blockade of Na^+/K^+-ATPase activity by Mg^{2+} ions was thought to be responsible for generating the K^+ concentration gradient and the subsequent ST segment changes.

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

ACKNOWLEDGMENTS. This work was supported by MEXT KAKENHI Grant, No. 16K08484, the Salt Science Research Foundation, No. 2028, the Tojuro Iijima Foundation for Food Science and Technology, No. 2019-12 and the Cooperative Study Program (19-305) of National Institute for Physiological Sciences to IK.

REFERENCES

1. Alaini, A., Roldan, C. A., Servilla, K. and Colombo, E. S. 2017. Near death by milk of magnesia. *BMJ Case Rep.* **2017**: 2017. [Medline]
2. Apell, H. J., Hitzler, T. and Schreiber, G. 2017. Modulation of the Na,K-ATPase by Magnesium Ions. *Biochemistry* **56**: 1005–1016. [Medline] [CrossRef]
3. Bara, M., Guiet-Bara, A. and Durlach, J. 1993. Regulation of sodium and potassium pathways by magnesium in cell membranes. *Magnes. Res.* **6**: 167–177. [Medline]
4. Cardozo, D. 2016. An intuitive approach to understanding the resting membrane potential. *Adv. Physiol. Educ.* **40**: 543–547. [Medline] [CrossRef]
5. Fuller, W., Parmar, V., Eaton, P., Bell, J. R. and Shattock, M. J. 2003. Cardiac ischemia causes inhibition of the Na/K ATPase by a labile cytosolic compound whose production is linked to oxidant stress. *Cardiovasc. Res.* **57**: 1044–1051. [Medline] [CrossRef]
6. Glitsch, H. G. 2001. Electrophysiology of the sodium-potassium-ATPase in cardiac cells. *Physiol. Rev.* **81**: 1791–1826. [Medline] [CrossRef]
7. Grycova, L., Sklenovsky, P., Lansky, Z., Janovska, M., Otyepka, M., Amler, E., Teisinger, J. and Kubala, M. 2009. ATP and magnesium drive conformational changes of the Na+/K+-ATPase cytoplasmic headpiece. *Biochim. Biophys. Acta* **1788**: 1081–1091. [Medline] [CrossRef]
8. Jhang, W. K., Lee, Y. J., Kim, Y. A., Park, S. J. and Park, Y. S. 2013. Severe hypermagnesemia presenting with abnormal electrocardiographic findings similar to those of hyperkalemia in a child undergoing peritoneal dialysis. *Korean J. Pediatr.* **56**: 308–311. [Medline] [CrossRef]
9. Kazama, I. 2016. Burn-induced subepicardial injury in frog heart: a simple model mimicking ST segment changes in ischemic heart disease. *J. Vet. Med. Sci.* **78**: 313–316. [Medline] [CrossRef]
10. Kazama, I. 2017. High-calcium exposure to frog heart: a simple model representing hypercalcemia-induced ECG abnormalities. *J. Vet. Med. Sci.* **79**: 71–75. [Medline] [CrossRef]
11. Kazama, I., Takamura, K., Yamada, Y., Sugisaki, Y. and Suzuki, M. 2020. Reciprocal ST segment changes reproduced in burn-induced subepicardial injury model in bullfrog heart. *J. Vet. Med. Sci.* **82**: 143–147. [Medline] [CrossRef]
12. Kléber, A. G. 2000. ST-segment elevation in the electrocardiogram: a sign of myocardial ischemia. *Cardiovasc. Res.* **45**: 111–118. [Medline] [CrossRef]
13. Kon, N., Abe, N., Miyazaki, M., Mushiake, H. and Kazama, I. 2018. Partial exposure of frog heart to high-potassium solution: an easily reproducible model mimicking ST segment changes. *J. Vet. Med. Sci.* **80**: 578–582. [Medline] [CrossRef]
14. Laurant, P. and Touyz, R. M. 2000. Physiological and pathophysiological role of magnesium in the cardiovascular system: implications in hypertension. *J. Hypertens.* **18**: 1177–1191. [Medline] [CrossRef]
15. Lorković, H. 1966. Influence of changes in pH on the mechanical activity of cardiac muscle. *Circ. Res.* **19**: 711–720. [Medline] [CrossRef]
16. Niedergerke, R. and Orkand, R. K. 1966. The dependence of the action potential of the frog's heart on the external and intracellular sodium concentration. *J. Physiol.* **184**: 312–334. [Medline] [CrossRef]
17. Nishikawa, M., Shimada, N., Kanzaki, M., Ikegami, T., Fukuoka, T., Fukushima, M. and Asano, K. 2018. The characteristics of patients with hypermagnesemia who underwent emergency hemodialysis. *Acute Med. Surg.* **5**: 222–229. [Medline] [CrossRef]
18. Pilotelle-Bunner, A., Cornelius, F., Sebban, P., Kuchel, P. W. and Clarke, R. J. 2009. Mechanism of Mg2+ binding in the Na+,K+-ATPase. *Biophys. J.* **96**: 3753–3761. [Medline] [CrossRef]
19. Pirkmajer, S. and Chibalin, A. V. 2016. Na,K-ATPase regulation in skeletal muscle. *Am. J. Physiol. Endocrinol. Metab.* **311**: E1–E31. [Medline] [CrossRef]
20. Wagner, G. S., Macfarlane, P., Wellens, H., Josephson, M., Gorgels, A., Mirvis, D. M., Pahlm, O., Surawicz, B., Kligfield, P., Childers, R., Gettes, L. S., Bailey, J. J., Deal, B. J., Gorgels, A., Hancock, E. W., Kors, J. A., Mason, J. W., Okin, P., Rautaharju, P. M., van Herpen G., American Heart Association Electrocardiography and Arrhythmias Committee, Council on Clinical Cardiology American College of Cardiology Foundation Heart Rhythm Society Endorsed by the International Society for Computerized Electrocardiology. 2009. AHA/ACCF/HRS recommendations for the standardization and interpretation of the electrocardiogram: part VI: acute ischemia/infarction: a scientific statement from the American Heart Association Electrocardiography and Arrhythmias Committee, Council on Clinical Cardiology; the American College of Cardiology Foundation; and the Heart Rhythm Society. *J. Am. Coll. Cardiol.* **53**: 1003–1011. [Medline] [CrossRef]
21. Wyskida, K., Witkowicz, J., Chudek, J. and Więcek, A. 2012. Daily magnesium intake and hypermagnesemia in hemodialysis patients with chronic kidney disease. *J. Ren. Nutr.* **22**: 19–26. [Medline] [CrossRef]