INVITED REVIEW ARTICLE

Nagoya J. Med. Sci. **63**. 91 ~ 98, 2000

PATHOGENESIS AND THE ROLE OF Ca^{2+} OVERLOAD DURING MYOCARDIAL ISCHEMIA/REPERFUSION

HIDEHARU HAYASHI

Department of Humoral Regulation,
Research Institute of Environmental Medicine,
Nagoya University

ABSTRACT

To study the regulation of $[Na^+]_i$ and $[Ca^{2+}]_i$ during myocardial ischemia/reperfusion, $[Na^+]_i$ and $[Ca^{2+}]_i$ were measured simultaneously using guinea pig ventricular myocytes which were dual-loaded with SBFI/AM and fluo-3/AM. It was suggested that: (1) $[Na^+]_i$ increased during metabolic inhibition (MI: 3.3 mM amytal and 5 μM CCCP) by both the activated Na^+ influx via Na^+/H^+ exchange and the suppressed Na^+ extrusion via the Na^+/K^+ pump; (2) Na^+/Ca^{2+} exchange was inhibited during MI, causing the dissociation between $[Na^+]_i$ and $[Ca^{2+}]_I$; (3) Na^+/Ca^{2+} exchange could be reactivated by energy repletion, resulting in a significant increase in $[Ca^{2+}]_i$, Furthermore, a Ca^{2+} influx via the reverse-mode of Na^+/Ca^{2+} exchange may play a key role in the mechanism of Ca^{2+} overload on reoxygenation; and (4) cell contracture during MI was related to rigor due to energy depletion, while cell contracture after energy repletion was likely to be related to Ca^{2+} overload.

Key Words: myocardial ischemia, reperfusion, $[Na^+]_i$, $[Ca^{2+}]_i$, Na^+/H^+ exchange, Na^+/K^+ pump, Na^+/Ca^{2+} exchange

INTRODUCTION

An abnormal handling of intracellular Ca^{2+} is supposed to be a final pathway of cell injury during myocardial ischemia/reperfusion. However, the mechanisms of Ca^{2+} overload and the relationship between such overload and cell injury in ischemia/reperfusion remain poorly defined.[1] Recent studies have indicated that intracellular Na^+ concentration ($[Na^+]_i$) increases during hypoxia/ischemia.[2-4] The elevated $[Na^+]_i$ leads, via Na^+/Ca^{2+} exchange, to the subsequent increase in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$), resulting in an inotropic or toxic effect.[5] However, it has been demonstrated in cardiac myocytes that the activity of Na^+/Ca^{2+} exchange is dependent on both the cellular ATP[6] and pH levels.[7] Therefore, it remains controversial whether the level of $[Ca^{2+}]_i$ is linked to that of $[Na^+]_i$ during ischemia/reperfusion and whether the increase in $[Ca^{2+}]_i$ is a cause of cell injury. If the interaction of $[Na^+]_i$ and $[Ca^{2+}]_i$ could be monitored in an isolated myocyte, it becomes possible to assess the regulating factors for Na^+/Ca^{2+} exchange activity at the cell level and their contribution to cell injury.

We have developed a new method for the simultaneous measurement of $[Na^+]_i$ and $[Ca^{2+}]_i$ in isolated guinea pig myocytes using sodium-binding benzofuran isophthalate (SBFI) and fluo-3 coupled with digital imaging fluorescence microscopy.[8-10] This technique can provide a great

Correspondence: Hideharu Hayashi, MD, Department of Humoral Regulation, Research Institute of Environmental Medicine, Nagoya University, Furo-cho, Chikusa-ku, Nagoya 464-8601, Japan
Tel: +81-52-789-5780 Fax: +81-52-789-5003 E-mail: hayashi@riem.nagoya-u.ac.jp

advantage to investigate the relationship between these ion concentrations. In this study, we monitored the relationships among $[Na^+]_i$, $[Ca^{2+}]_i$ and cell shape during metabolic inhibition (MI) and energy repletion (ER). For MI,[11),12)] the perfusate contained 3.3 mM amytal and 5 μM carbonyl cyanide m-chlorophenylhydrazone (CCCP) in the absence of glucose.[12)] For energy repletion (ER), 10 mM glucose was applied for 30 min in the presence of metabolic inhibitors after 20 min of MI.[11)]

REGULATION OF $[Na^+]_i$, $[Ca^{2+}]_i$ AND CELL SHAPE DURING METABOLIC INHIBITION

During the early phase of MI, 3 possible Na^+ transport pathways have been implicated to account for the increase in $[Na^+]_i$: (1) increased Na^+ influx via Na^+/H^+ exchange activated by glycolysis-induced intracellular acidosis,[2)] (2) Na^+ influx via the fast Na^+ channel,[3)] and (3) decreased Na^+ extrusion via the Na^+/K^+ pump.[4)]

The time courses of the changes in $[Na^+]_i$ during 30 min perfusion of metabolic inhibitors in the absence of glucose, or during 30 min of MI in the presence of 1 μM hexamethylene amiloride (HMA), a specific inhibitor of Na^+/H^+ exchange,[13)] or 10 μM tetrodotoxin (TTX), an inhibitor of the fast Na^+ channel were examined. Cells were preincubated with these agents for at least 10 min before MI. During MI, the application of HMA suppressed the increase in $[Na^+]_i$ significantly, whereas TTX had no effect. The rise of $[Na^+]_i$ by the perfusion of metabolic inhibitors was also suppressed by the addition of glucose. The values of $[Na^+]_i$ at 30 min were 25.0±2.4 mM in MI only, 12.3±1.3 mM in the presence of HMA, and 28.1±6.3 mM in the presence of TTX, respectively.[11)]

The involvement of Na^+/H^+ exchange was supported by our observation that HMA prevented the increase in $[Na^+]_i$ during the early phase of MI. The involvement of the Na^+ channel is unlikely in our study, because we used resting, unstimulated myocytes. In fact, 10 μM TTX could not suppress the increase in $[Na^+]_i$ during MI.[14)]

When myocytes were exposed to a K^+-free solution, which would inhibit the Na^+/K^+ pump, $[Na^+]_i$ increased from 6.7±0.2 mM to 25.9±1.4 mM ($p<0.01$) during normoxic perfusion, and from 6.7±0.5 mM to 33.3±2.7 mM ($p<0.01$) during MI.[15)] The readmission of 5 mM K^+, which reactivates the Na^+/K^+ pump, could partly reverse $[Na^+]_i$ during normoxic perfusion, but could not reverse $[Na^+]_i$ during MI. It was suggested that the Na^+/K^+ pump was significantly inhibited after 10 min of MI. It is likely that ATP_i depletion during MI could cause the time-dependent inhibition of the Na^+/K^+ pump, resulting in a gradual increase in $[Na^+]_i$.

Next, we investigated the relationships among $[Na^+]_i$, $[Ca^{2+}]_i$ and cell shape during MI, and compared with those during strophanthidin (digitalis) perfusion as illustrated in Fig. 1. During the perfusion of 500 μM strophanthidin, both $[Na^+]_i$ and $[Ca^{2+}]_i$ increased significantly in precontracted (rod-shaped) cells. The increases in $[Na^+]_i$ and $[Ca^{2+}]_i$ became greater as the cell shape changed to contracted and hypercontracted forms.[9)]

On the other hand, there was no increase in $[Ca^{2+}]_i$ in precontracted cells and contracted cells during MI, though $[Na^+]_i$ increased significantly (Fig. 1). When cells hypercontracted, $[Ca^{2+}]_i$ increased significantly. The $[Ca^{2+}]_i$ levels during MI were much lower than those during strophanthidin perfusion ($p<0.01$).[11)] These data suggested that although $[Na^+]_i$ and $[Ca^{2+}]_i$ increased significantly during MI, the distinct increase in $[Ca^{2+}]_i$ occurred after myocytes had hypercontracted, and that there was a dissociation in the relationship between $[Na^+]_i$ and $[Ca^{2+}]_i$.

In this study, we estimated the activity of Na^+/Ca^{2+} exchange from the relationship between $[Na^+]_i$ and $[Ca^{2+}]_i$ in intact myocytes, and suggested that the activity was suppressed during MI.

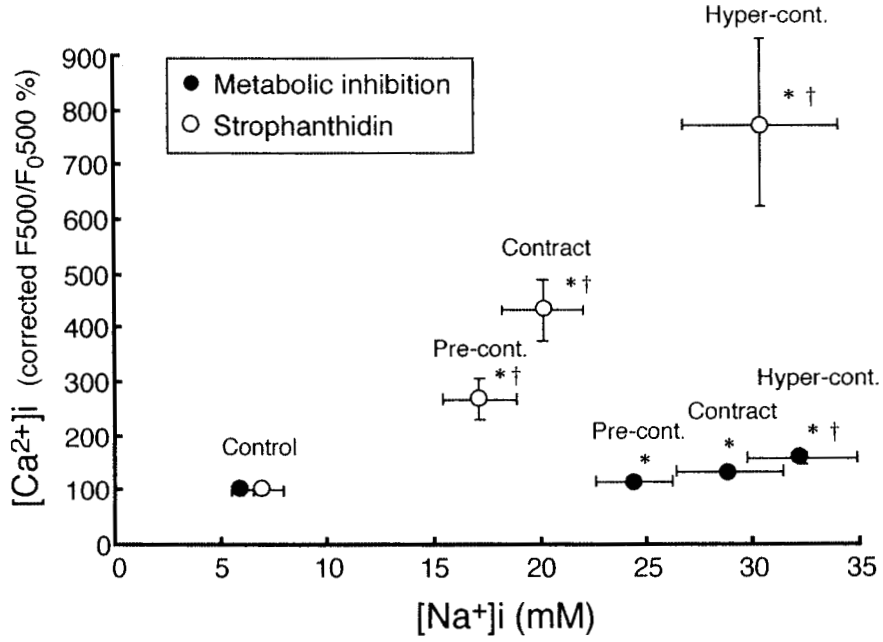

Fig. 1. Relationships among cell shape, $[Na^+]_i$ and $[Ca^{2+}]_i$. The relationships among cell shape, $[Na^+]_i$ and $[Ca^{2+}]_i$ were compared between during metabolic inhibition (●; n=31) and during 500 μM strophanthidin perfusion (○; n=20). Control: rod-shaped cells before perfusion. Pre-cont.: rod-shaped cells 1–5 min before contracture. Contract: contracted cells. Hyper-cont.: hypercontracted cells. Values are means±SE. * $p<0.01$ vs control in $[Na^+]_i$, and † $p<0.01$ vs control in $[Ca^{2+}]_i$ by one-way ANOVA.[11]

Miura and Kimura[16] revealed that the increase in $[Na^+]_i$ would shift the reversal potential of Na^+/Ca^{2+} exchange toward more negative membrane potentials. Though this shift would thereby promote Ca^{2+} influx via the reverse-mode of Na^+/Ca^{2+} exchange, there was no significant increase in $[Ca^{2+}]_i$ during MI in our study. The ATP-dependence of Na^+/Ca^{2+} exchange has been reported in cardiac vesicles,[17] giant excised cardiac sarcolemmal patches[6] and cardiac myocytes.[18] Doering and Lederer[7] also showed that protons inhibit Na^+/Ca^{2+} exchange. It is, therefore, likely that the dissociation between $[Na^+]_i$ and $[Ca^{2+}]_i$ during MI was related to the inhibition of Ca^{2+} entry via Na^+/Ca^{2+} exchange by both ATP depletion and intracellular acidosis.

Fig. 2 summarizes the effects of various agents on the changes in the % of rod-shaped cells, $[Na^+]_i$ and $[Ca^{2+}]_i$ during 50 min of MI. The perfusion of 1 μM HMA completely abolished the increase in $[Na^+]_i$ but did not affect the increase in $[Ca^{2+}]_i$ nor the % of rod-shaped cells. This finding implies that the increase in $[Ca^{2+}]_i$ was independent of the increase in $[Na^+]_i$. In other words, Ca^{2+} influx via Na^+/Ca^{2+} exchange (or reduced Ca^{2+} extrusion via the exchange) was not responsible for the increase in $[Ca^{2+}]_i$. Therefore, the increase in $[Ca^{2+}]_i$ could be related to the depression of other regulatory mechanisms of $[Ca^{2+}]_i$, such as sarcolemmal Ca^{2+}-ATPase and/or SR Ca^{2+} uptake. When Ca^{2+}-free solution (with 1 mM EGTA) was perfused (0Ca), $[Ca^{2+}]_i$ was significantly lower than that in MI, but the decrease in the % of rod-shaped cells was not attenuated. These data suggested that the increase in $[Na^+]_i$ or $[Ca^{2+}]_i$ was not a prerequisite for cell contracture during MI. When 10 mM glucose was perfused with metabolic inhibitors, cells could remain viable using glycolytic energy.[19] After 50 min perfusion of metabolic inhibitors in the presence of 10 mM glucose (10G MI), only 2 of 28 myocytes developed contracture. The

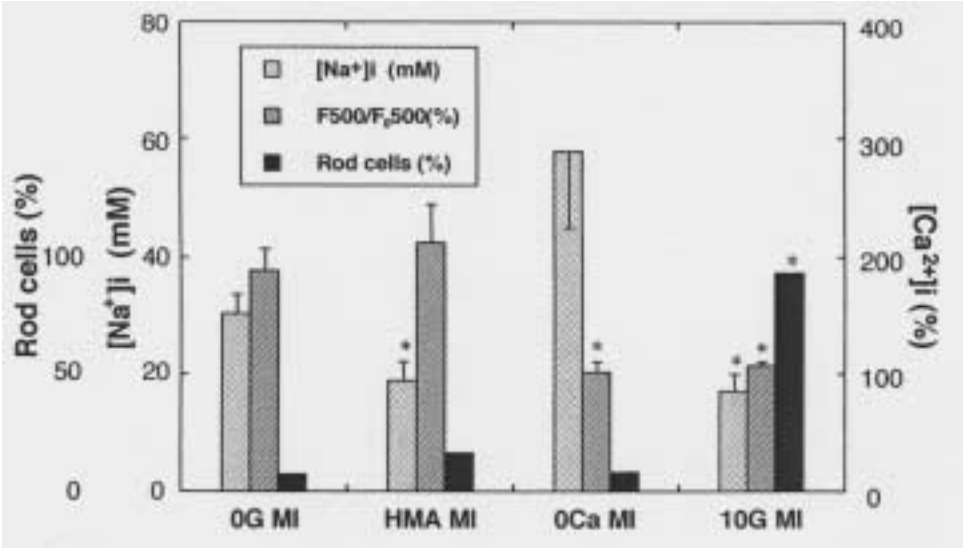

Fig. 2. Factors contributing to changes in cell shape, $[Na^+]_i$ and $[Ca^{2+}]_i$ during MI. MI was achieved by applying 3.3 mM amytal and 5 μM CCCP for 50 min in glucose-free Krebs solution (0G MI). The effects of 50 min perfusion of 1 μM hexamethylene amiloride (HMA), Ca^{2+}-free solution (0Ca), or 10 mM glucose (10G) on the increases in $[Na^+]_i$, $[Ca^{2+}]_i$ (expressed as corrected F500/F0500), and on the % of rod-shaped cells, were examined. Values are means±SE and compared with those in 0G MI. * $p<0.01$ by one-way ANOVA, or $p<0.05$ by chi-squared test.[11]

increases in $[Na^+]_i$ and $[Ca^{2+}]_i$ were much less than those during MI.

We showed that cell contracture during MI was not related to Ca^{2+} overload but was related to rigor due to energy depletion. In fact, contracture developed before a significant increase in $[Ca^{2+}]_i$ occurred and was not protected by the perfusion of Ca^{2+}-free solution.[20] In contrast, the presence of 10 mM glucose was effective for the preservation of rod-shaped cells.[19] It was concluded that energy depletion could be a main cause of both cell contracture and the changes in ionic concentrations during MI.

EFFECTS OF ENERGY REPLETION ON $[Na^+]_i$, $[Ca^{2+}]_i$ AND CELL SHAPE

Fig. 1 showed a dissociation in the relationship between $[Na^+]_i$ and $[Ca^{2+}]_i$ during MI compared with that during strophanthidin perfusion. If the energy depletion was a cause of this dissociation, energy repletion could cancel it. After 20 min of MI, 10 mM glucose was applied for 30 min in the presence of metabolic inhibitors (energy repletion: ER). After 20 min of MI, $[Na^+]_i$ increased from 6.4±0.3 mM to 16.0±1.2 mM, whereas $[Ca^{2+}]_i$ was kept at a low level. After ER, $[Na^+]_i$ continued to increase, reaching 42.6±4.8 mM at 50 min (Fig. 3; the left column). $[Ca^{2+}]_i$ showed a marked progressive increase to 442±72% of the control at 50 min ($p<0.01$ v.s. in MI). Furthermore, these changes were accompanied by the morphological changes from rod-shaped form to contracted and hypercontracted forms. The % of rod-shaped cells decreased significantly at 50 min (the far left column of Fig. 3). It was suggested that ER could, at least in part, eliminate the dissociation between $[Na^+]_i$ and $[Ca^{2+}]_i$.[11] As the pH_i levels were similar before and after the addition of glucose (data not shown), ER by glycolysis was

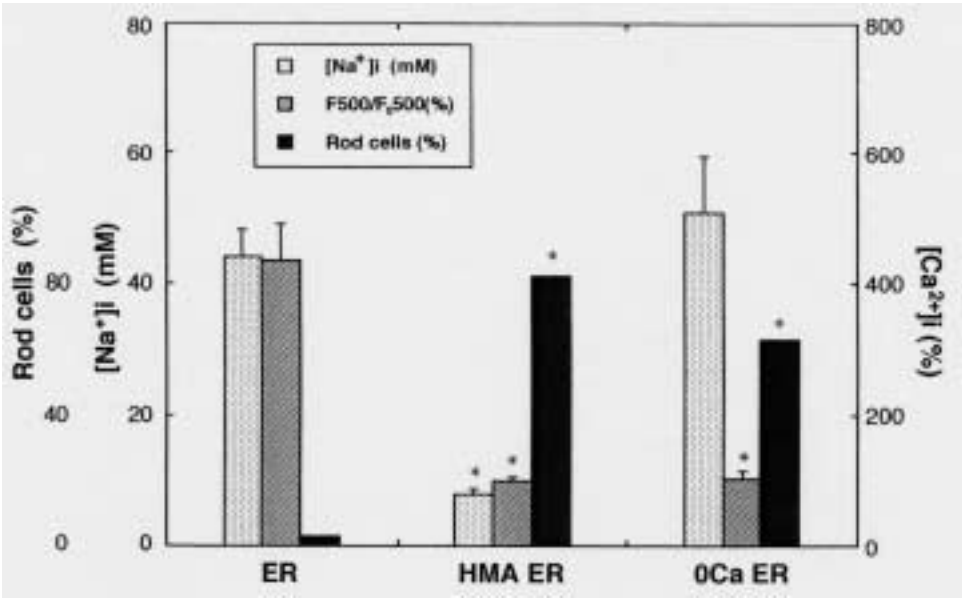

Fig. 3. Effects of energy repletion (ER) on the changes in cell shape, $[Na^+]_i$ and $[Ca^{2+}]_i$. After 20 min of MI, 10 mM glucose was applied for 30 min (ER). The effects of the perfusion of 1 μM HMA or Ca^{2+}-free solution (0Ca) on the increases in $[Na^+]_I$, $[Ca^{2+}]_i$ (expressed as corrected F500/F0500), and on the % of rod-shaped cells were examined. Drugs were present at least 10 min before MI and during entire periods of MI and ER. Values are means±SE and compared with those in ER only. * $p<0.01$ by one-way ANOVA, or $p<0.05$ by chi-squared test.[11)]

thought to be a main cause of the reactivation of the Ca^{2+} entry via Na^+/Ca^{2+} exchange. Haworth and Gokner[18)] have described that as the calculated Km of Na^+/Ca^{2+} exchange for ATP was no more than 10% of normal ATP level, the exchanger would not be limited by ATP as long as it kept 25% of the original level.

Next, we sought to examine the contribution of $[Na^+]_i$ and extracellular Ca^{2+} to the increase in $[Ca^{2+}]_i$ and cell shape after ER. Fig. 3 also summarizes alterations in the % of rod-shaped cells, $[Na^+]_i$ and $[Ca^{2+}]_i$ after ER in the presence of 1 μM HMA or Ca^{2+}-free solution (0Ca). Cells were perfused with HMA or Ca^{2+}-free solution during the overall period of MI and ER. HMA suppressed the decrease in the % of rod-shaped cells and the increases in both $[Na^+]_i$ and $[Ca^{2+}]_i$ after ER. In the Ca^{2+}-free perfusion (0Ca), although $[Na^+]_i$ increased further, both the decrease in the % of rod-shaped cells and the increase in $[Ca^{2+}]_i$ were suppressed. Since the suppression of the increase in $[Na^+]_i$ (by HMA) and the removal of external Ca^{2+} could nearly eliminate the increase in $[Ca^{2+}]_i$ and cell contracture, it was suggested that the increase in $[Ca^{2+}]_i$ after ER was dependent on the increased Ca^{2+} influx (or decreased Ca^{2+} efflux) via Na^+/Ca^{2+} exchange, and that cell contracture was, at least in part, related to the increase in $[Ca^{2+}]_i$. Previous studies have also demonstrated that the manipulation of $[Na^+]_i$ during ischemia could influence postischemic Ca^{2+} overload and myocardial dysfunction, suggesting the contribution of Na^+/Ca^{2+} exchange.[21)]

In this study, cell contracture after ER was associated with the increase in $[Ca^{2+}]_i$, and it is now accepted that Ca^{2+} overload precipitates many different processes which cause cell damage, including uncoupling of mitochondrial oxidative phosphorylation, activation of phospholipases, production of free fatty acid and lysophospholipids and activation of Ca^{2+}-ATPase.[22)]

ROLE OF REVERSE-MODE OF Na^+/Ca^{2+} EXCHANGE ON REOXYGENATION-INDUCED ARRHYTHMIA

We have reported previously that triggered activities due to delayed afterdepolarizations (DADs) are elicited on reoxygenation after 60 min substrate-free hypoxia in guinea pig papillary muscles.[23] DADs and aftercontractions are related to the oscillatory Ca^{2+} releases from the sarcoplasmic reticulum (SR), and are observed in Ca^{2+} overload. As discussed earlier, a possible candidate for Ca^{2+} overload is a Ca^{2+} influx via the reverse-mode of Na^+/Ca^{2+} exchange. The major problem in the study of Na^+/Ca^{2+} exchange was that there was no specific inhibitor of the exchanger.

We studied the effects of a novel agent reported to selectively block Ca^{2+} influx by Na^+/Ca^{2+} exchange, KB-R7943 (2-[2-[4-(4-nitrobenzyloxy) phenyl]ethyl]isothiourea methanesulfonate),[24] in rat ventricular myocytes loaded with indo-1. KB-7943 dose-dependently inhibited the rise in $[Ca^{2+}]_i$ induced by rapid switch to a Na^+-free solution (9.9±6.6% of control at 5 μM), but it did not affect the rate of twitch $[Ca^{2+}]_i$ decline when the SR function was completely abolished by thapsigargin and ryanodine.[25] This indicates that 5–10 μM KB-7943 selectively blocks Ca^{2+} entry via Na^+/Ca^{2+} exchange (and not Ca^{2+} efflux).

Fig. 4 (the upper record) shows the representative slow speed record of contractile response of the control muscle. During 60 min substrate-free hypoxia, there was a decline in developed tension and an increase in resting tension. Within minutes of subsequent reoxygenation, the arrhythmia associated with the occurrence of aftercontractions was elicited as shown in the inset. Fig. 4 (the lower record) shows the record in the muscle treated with KB-R7943 (10 μM). In this muscle, there was no arrhythmia after reoxygenation. All of 7 control muscles showed reoxygenation-induced arrhythmia, whereas there was no arrhythmia in 3 of 7 muscles treated with KB-R7943. KB-R7943 also decreased the amplitudes of aftercontractions ($p<0.05$), and increased the recovery of developed tension 30 min after reoxygenation ($p<0.05$).[26]

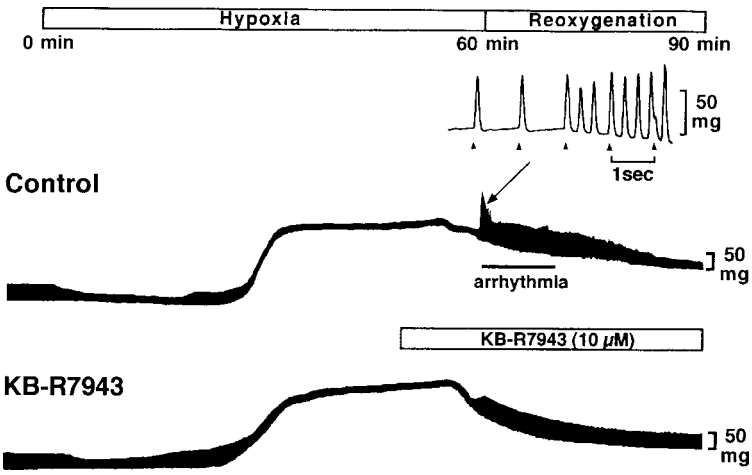

Fig. 4. The representative slow speed record of contractile response of guinea pig papillary muscles reoxygenated after 60 min substrate-free hypoxia. The muscles were stimulated at 1 Hz. The upper trace illustrates the record of control muscle. The faster speed record shows that arrhythmia was observed within minutes of reoxygenation. The lower record is of the muscle treated with 10 μM KB-R7943. KB-R7943 was applied 10 min before reoxygenation, and the muscle was reoxygenated in the presence of KB-R7943.[26]

In conclusion, it is likely that KB-R7943 selectively inhibits the reverse-mode of Na^+/Ca^{2+} exchange, and that it attenuates reoxygenation-induced arrhythmia in guinea pig papillary muscles. We also found that KB-R7943 significantly enhances post-hypoxia recovery of developed tension. These results suggest that Ca^{2+} influx via the reverse-mode of Na^+/Ca^{2+} exchange may play a key role in the mechanism of Ca^{2+} overload on reperfusion.

CONCLUSION

We investigated the relationship between intracellular ion concentrations during metabolic inhibition/energy repletion using a single cell model. During myocardial ischemia, $[Na^+]_i$ increases by both the activated Na^+ influx via Na^+/H^+ exchange and the suppressed Na^+ extrusion via the Na^+/K^+ pump. However, Na^+/Ca^{2+} exchange is inhibited by energy depletion and intracellular acidosis, causing the dissociation between $[Na^+]_i$ and $[Ca^{2+}]_i$. After reperfusion, Na^+/H^+ exchange is activated further as pH_i is recovered, resulting in an additional $[Na^+]_i$ elevation. Na^+/Ca^{2+} exchange could be reactivated by reperfusion, resulting in a significant increase in $[Ca^{2+}]_i$, and a Ca^{2+} influx via the reverse-mode of Na^+/Ca^{2+} exchange may play a key role in the mechanism of Ca^{2+} overload on reperfusion. It was also suggested that cell contracture during ischemia is related to rigor due to energy depletion, while cell contracture after reperfusion is likely to be related to Ca^{2+} overload.

REFERENCES

1) Allen, D.G. and Orchard, C.H.: Myocardial contractile function during ischemia and hypoxia. *Circ. Res.*, 60, 153–168 (1987).
2) Lazdunski, M., Frelin, C. and Vigne, P.: The sodium/hydrogen exchange system in cardiac cells: its biochemical and pharmacological properties and its role in regulating internal concentrations of sodium and internal pH. *J. Mol. Cell. Cardiol.*, 17, 1029–1042 (1985).
3) January, C.T. and Fozzard H.A.: The effects of membrane potential, extracellular potassium, and tetrodotoxin on the intracellular sodium ion activity of sheep cardiac muscle. *Circ. Res.*, 54, 652–664 (1984).
4) Guarnieri, T.: Intracellular sodium-calcium dissociation in early contractile failure in hypoxic ferret papillary muscle. *J. Physiol. Lond.*, 388, 449–465 (1987).
5) Sheu, S.S. and Fozzard, H.A.: Transmembrane Na^+ and Ca^{2+} electrochemical gradients in cardiac muscle and their relationship to force development. *J. Gen. Physiol.*, 80, 325–351 (1982).
6) Collins, A., Somlyo, A.V. and Hilgemann, D.W.: The giant cardiac membrane patch method: stimulation of outward Na^+-Ca^{2+} exchange current by Mg ATP. *J. Physiol. Lond.*, 454, 27–57 (1992).
7) Doering, A.E. and Lederer, W.J.: The mechanism by which cytoplasmic protons inhibit the sodium-calcium exchanger in guinea-pig heart cells. *J. Physiol. Lond.*, 466, 481–499 (1993).
8) Hayashi, H., Satoh, H., Noda, N., Terada, H., Kobayashi, A., Hirano, M., Yamashita, Y. and Yamazaki, N.: Simultaneous measurement of intracellular Na^+ and Ca^{2+} during K^+-free perfusion in isolated myocytes. *Am. J. Physiol.*, 266, C416–C422 (1994).
9) Satoh, H., Hayashi, H., Noda, N., Terada, H., Kobayashi, A., Hirano, M., Yamashita, Y. and Yamazaki, N.: Regulation of $[Na^+]_i$ and $[Ca^{2+}]_i$ in guinea pig myocytes: dual loading of fluorescent indicators SBFI and fluo 3. *Am. J. Physiol.*, 266, H568–H576 (1994).
10) Hayashi, H. and Miyata, H.: Fluorescence imaging of intracellular Ca^{2+}. *J Pharmacol. Toxicol.*, 31, 1–10 (1994).
11) Satoh, H., Hayashi, H., Katoh, H., Terada, H. and Kobayashi, A.: Na^+/H^+ and Na^+/Ca^{2+} exchange in regulation of $[Na^+]_i$ and $[Ca^{2+}]_i$ during metabolic inhibition. *Am. J. Physiol.*, 268, H1239–H1248 (1995).
12) Li, Q., Hohl, C.M., Altshuld, R.A. and Stokes, B.T.: Energy depletion-repletion and calcium transients in single cardiomyocytes. *Am. J. Physiol.*, 257, C427–C434 (1989).
13) Dennis, S.C., Coetzee, W.A., Cragoe, Jr. E.J. and Opie, L.H.: Effects of proton buffering and of amiloride

derivatives on reperfusion arrhythmias in isolated rat hearts. *Circ. Res.*, 66, 1156–1159 (1990).
14) MacLeod, K.T.: Effects of hypoxia and metabolic inhibition on the intracellular sodium activity of mammalian ventricular muscle. *J. Physiol. Lond.*, 416, 455–468 (1989).
15) Katoh, H., Satoh, H., Nakamura, T., Terada, H. and Hayashi, H.: The role of Na^+/H^+ exchange and the Na^+/K^+ pump in the regulation of $[Na^+]i$ during metabolic inhibition in guinea pig myocytes. *Biochem. Biophys. Res. Commun.*, 203, 93–98 (1994).
16) Miura, Y. and Kimura, J.: Sodium-calcium exchange current: dependence on internal Ca and Na and competitive binding of external Na and Ca. *J. Gen. Physiol.*, 93, 1129–1145 (1989).
17) Caroni, P. and Carafoli. E.: The regulation of the Na^+-Ca^{2+} exchanger of heart sarcolemma. *Eur. J. Biochem.*, 132, 451–460 (1983).
18) Howorth, R.A. and Gokner, A.B.: ATP dependence of calcium uptake by the Na-Ca exchanger of adult heart cells. *Circ. Res.*, 71, 210–217 (1992).
19) Hayashi, H., Miyata, H., Noda, N., Kobayashi, A., Hirano, M., Kawai, T. and Yamazaki, N.: Intracellular Ca^{2+} concentration and pH_i during metabolic inhibition. *Am. J. Physiol.*, 262, C628–C634 (1992).
20) Li, Q., Altshuld, R.A. and Stokes, B.T.: Myocyte deenergization and intracellular free calcium dynamics. *Am. J. Physiol.*, 255, C162–C168 (1988).
21) Tani, M. and Neely, J.R.: Role of intracellular Na^+ in Ca^{2+} overload and depressed recovery of ventricular function of reperfused ischemic rat heart. *Circ. Res.*, 65, 1045–1056 (1989).
22) Cheung, J.Y., Bonventre, J.V., Malis, C.D. and Leaf, A.: Calcium and ischemic injury. *New Engl. J. Med.*, 314, 1670–1676 (1986).
23) Hayashi, H., Ponnambalam, C. and McDonald, T.F.: Arrhythmic activity in reoxygenated guinea pig papillary muscles and ventricular cells. *Circ. Res.*, 61, 124–133 (1987).
24) Iwamoto, T., Watano, T. and Shigekawa, M.: A novel isothiourea derivative selectively inhibits the reverse mode of Na^+/Ca^{2+} exchange in cells expressing NCX1. *J. Biol. Chem.*, 271, 22391–22397 (1996).
25) Satoh, H., Ginsburg, K.S., Qing, K., Terada, H., Hayashi, H. and Bers, D.M.: KB-R7943 block of Ca^{2+} influx via Na^+/Ca^{2+} exchange does not alter twitches or glycoside inotropy, but prevents Ca^{2+} overload in rat ventricular myocytes. *Circulation*, 101, 1441–1446 (2000).
26) Mukai, M., Terada, H., Sugiyama, S., Satoh, H., Hayashi, H. and Ohno, R.: Effects of a selective inhibitor of Na^+/Ca^{2+} exchange, KB-R7943, on reoxygenation-induced injuries in guinea pig papillary muscles. *J. Cardiovasc. Pharmacol.*, 35, 121–128 (2000).