Yonsei Medical Journal
Vol. 36, No. 2, 1995

Intracellular Acidosis Decreases the Outward Na^+-Ca^{2+} Exchange Current in Guinea Pig Ventricular Myocytes

Ek Ho Lee, So Ra Park, *Kwang Se Paik and Chang Kook Suh

The Na^+-Ca^{2+} exchange transport operating in outward mode has been suggested to cause Ca^{2+} entry during reperfusion or reoxygenation, exchanging extracellular Ca^{2+} for intracellular Na^+ that has accumulated during ischemia or cardioplegia. During cardioplegia, however, an increase in Ca^{2+} entry via this mechanism can be decreased due to increased intracellular H^+ activity and a decrease in cellular ATP content. In this study giant excised cardiac sarcolemmal membrane patch clamp technique was employed to investigate the effect of cytosolic pH change on the Na^+-Ca^{2+} exchanger, excluding the effect of ATP, in guinea pig cardiac myocytes. The outward Na^+-dependent current, which has a characteristics of Hill equation, was decreased as pH was decreased in the range of 7.5 ~6.5. The current density generated by the Na^+-Ca^{2+} exchange transport was 56.6±4.4 pA/pF (Mean±S.E.M.) at pH 7.2 and decreased to 42.9±3.0 pA/pF at pH 6.9. These results imply that Na^+-Ca^{2+} exchange transport, operating in a reverse mode during cardioplegia, decreases due to increased intracellular H^+, and further suggest that consequent intracellular Na^+ accumulation is one of aggravating factors for Ca^{2+} influx during reoxygenation or reperfusion.

Key Words: Na^+-Ca^{2+} exchange, intracellular pH, cardioplegia, ischemia, acidosis

Free calcium (Ca^{2+}) entry during reperfusion or reoxygenation has been considered as one of the causes of cardiac injury (calcium paradox) after cardioplegia (Shen & Jennings, 1972; Hearse, 1977; Hearse *et al* 1978; Nayler 1981). Among the various pathways, the Na^+-Ca^{2+} exchange transport operating in reverse mode is suggested as one of main mechanisms for Ca^{2+} influx (Renlund *et al.*, 1984; Murphy *et al.* 1988; Tani, 1990; Tani & Neely, 1990a; Weiss *et al.* 1990).

During the ischemic cardiac arrest, intracellular Na^+ activity increases (Grinwald, 1982; Jennings *et al.* 1985; Lazdunski *et al.* 1985; Fiolet *et al.* 1986; Pike *et al.* 1990; Tani & Neely, 1990a; Kim *et al.* 1993), and increased Na^+ activity provides the driving force for Na^+-Ca^{2+} exchanger to transport extracellular Ca^{2+} into the sarcoplasm (Renlund *et al.* 1984; Murphy *et al.* 1988; Weiss *et al.* 1990; Tani & Neely, 1990a,b). These reports provide evidences that intracellular Na^+ accumulation during ischemic cardiac arrest is one of main factors for the calcium paradox (Tani, 1990; Kim *et al.* 1993). Possible factors of increasing intracellular Na^+ activity with sustained depolarization include Na^+ background current, Na^+-H^+ exchange transport, and Na^+-Ca^{2+} exchange

Received February 13, 1995
Accepted April 26, 1995
Department of Physiology, Inha University College of Medicine, Inchon, *Department of Physiology, Yonsei University College of Medicine, Seoul, KOREA
Address reprint requests to Dr. C.K. Suh, Department of Physiology, Inha University College of Medicine, #253, Yong Hyun-Dong, Nam-Ku, Inchon 402-751 KOREA
This study was supported by the 1993 year program of the Inha University Research Funding and the Research Grant (#931-0700-004-2) from Korea Science and Engineering Foundation(KOSEF).

transport (Mullins, 1981; Ahn *et al.* 1987; Hagiwara *et al.* 1992; Kiyosue *et al.* 1992). However, the mechanism of intracellular Na^+ accumulation is not clearly understood.

Intracellular H^+ activity increases due to anaerobic glycolysis and ATP consumption during ischemic cardiac arrest (Mullins, 1977; Philipson *et al.* 1982; Haworth *et al.* 1987). And decreased intracellular pH could have effects on transmembrane ionic movements including the Na^+-Ca^{2+} exchanger and the Na^+-H^+ exchanger. Impairment of the Na^+-Ca^{2+} exchanger (in reverse mode) would certainly enhance intracellular Na^+ accumulation.

There have been some studies on the effect of pH on Na^+-Ca^{2+} exchange using cardiac sarcolemmal vesicles (Philipson *et al.* 1982) and whole cell patch clamp with internal dialysis (Earm & Irisawa, 1986). However, there were some limitations in interpreting the results of these studies (Reeves & Philipson, 1989). Recently, a method called giant excised cardiac sarcolemmal membrane patch technique (Hilgemann, 1989) was developed, which is believed to minimize experimental limitations and very useful in studying Na^+-Ca^{2+} exchange transport.

Thus, to investigate the effect of intracellular acidosis on the Na^+-Ca^{2+} exchange transport during ischemic cardiac arrest, giant excised cardiac sarcolemmal membrane patch technique was employed to measure the effect of pH on the currents generated by the Na^+-Ca^{2+} exchanger.

METHODS

Giant excised cardiac sarcolemmal membrane patch method (Hilgemann, 1989) was used to measure the effect of pH on the Na^+-Ca^{2+} exchange current. Guinea pig ventricular myocytes were isolated by a standard retrograde Langendorff perfusion method, recirculating low Ca^{2+} Tyrode solution (120 mM NaCl, 5.4 mM KCl, 5 mM nitrilotriacetic acid, 5 mM pyruvate, 20 mM glucose, 20 mM taurine, 10 mM N-[2-hydroxyethyl] piperazine-N'-[2-ethanesulfonic acid] (HEPES), 12 μM) free Ca^{2+}, pH 7.0) and 0.04 mg/ml collagenase (Sigma type I) solution (same as low Ca^{2+} Tyrode solution differing only in free Ca^{2+} concentration: 50 μM) for 5 and 20 minutes, respectively. Estimation of free Ca^{2+} concentrations in the solutions was made according to Fabiato (1981).

The isolated cells were then incubated in high KCl/zero calcium solution to induce separation of the surface membrane from the underlying structure. After about 3 hours of incubation, "blebs" in diameter of 10~30 μm were formed on cells with little or no cell shortening. Glass electrodes (Metex capillary tubes 150×1.3 mm ID, wall 0.2 mm, Mercer glass works, INC) with a tip diameter of 5~9 μm were prepared with a two-stage pipette puller (List Electronic, L/M-3P-A) and were fire polished with a microforge (Narishige, MF-83). The prepared glass electrode was attached on the "bleb" to make a seal. And inside-out patch was formed after a short period of air exposure. Solutions of various ion concentrations were perfused at room temperature and the resulting currents were recorded using an amplifier (Axopatch 200, Axon Instruments) on a chart recorder (Harvard, Universal Oscillograph). In order to transform the detected currents into current densities, capacitance compensation method was used to measure patch membrane capacitance.

Electrode pipettes were filled with a solution containing 25 mM CsCl, 25 mM tetraethyl ammonium (TEA), 1 mM 4-aminopyridine, 1 mM $BaCl_2$, 10 μM verapamil, 25 μM ouabain, 5 mM $CaCl_2$, 5 mM KCl, 100 mM N-methylglucamine (NMG) at pH 7.2. Experimental bath, staged on inverted microscope, was perfused with solutions containing 25 mM CsCl, 25 mM TEA, 5 mM KCl, 25 mM HEPES, 20 mM ethyleneglycol-bis-[β-aminoethyl ether] N, N, N' N'-tetraacetic acid (EGTA), 6 mM $CaCO_3$, 1 mM $MgCl_2$, and various concentrations of NaCl and LiCl added to make the total concentration of 100 mM at pH 7.2. To measure pH effect on the outward currents, the experimental bath was perfused with solutions containing 25 mM CsCl, 25 mM TEA, 5 mM KCl, 25 mM HEPES, 20 mM EGTA, 6 mM $CaCO_3$, 1 mM $MgCl_2$, 100 mM NaCl, and

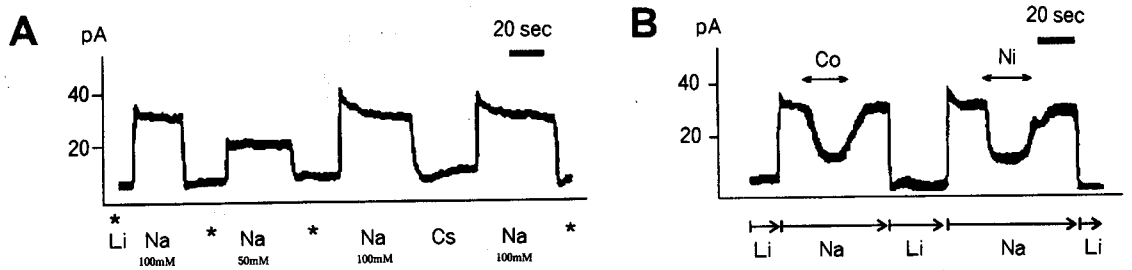

Fig. 1. *Measurements of Na^+-Ca^{2+} exchange current using the giant excised cardiac sarcolemmal membrane patch technique. A: Typical recordings of the Na^+-Ca^{2+} exchange current while the bath was perfused with solutions containing 100 mM NaCl (100 Na), 50 mM NaCl and 50 mM LiCl (50 Na), 100 mM LiCl (Li,*) or 100 mM CsCl (Cs), B: blockage of the Na^+-Ca^{2+} exchange current when perfused with solutions containing Co^{2+} 2 mM (Co) or Ni^{2+} 5 mM (Ni) in the presence of NaCl 100 mM.*

the pH of solutions were titrated to 7.5, 7.2, 6.9, and 6.5 with NMG.

RESULTS

Fig. 1 shows evidences of the Na^+-Ca^{2+} exchange current operating in reverse mode, after blocking Na^+-K^+ pump activity and the ionic currents through K^+ channel and Ca^{2+} channel (see METHODS for detailed solution content) at a 0 mV holding potential. When perfused with solutions containing 100 mM Na^+ on the cytoplasmic side of the excised patch, in the presence of Ca^{2+} on the extracellular side, an outward current was recorded. This outward current was not detected when Na^+ was replaced with Li^+ or Cs^+ (Fig. 1-A). After restoring the outward current with perfusion of 100 mM Na^+ solution, the patch was perfused with 50 mM Na^+ solution and the outward current was decreased. This Na^+-dependent outward current was partially blocked by 1 mM Co^{2+} or 5 mM Ni^{2+}, inhibitors of Na^+-Ca^{2+} exchanger (Fig. 1-B).

The recorded currents were transformed into current densities to normalize Na^+-Ca^{2+} exchange currents measured from the different patch sizes. The average of 26 measurements at 100 mM $[Na^+]_i$ and 30 nM $[Ca^{2+}]_o$ was 61.2±8.7 pA/pF (Mean±S.E.M.) at pH 7.2. As Na^+ concentration was reduced to 75, 50,

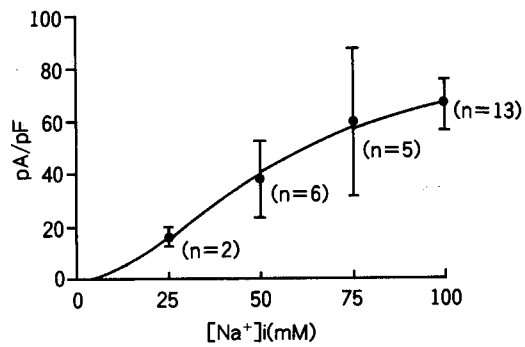

Fig. 2. *Na^+-dependency of the Na^+-Ca^{2+} exchange current. Currents are transformed into current densities after measuring patch membrane capacitance. Line fitted with Fig P. (BIOSOFT®, Cambridge, UK) has a Hill coefficient of 2.0 and a half saturation of 52 mM.*

and 25 mM, the current density was decreased to 59.5±28.1 pA/pF (n=5), 37.9±14.6 (n=6) pA/pF, and 16.4±3.8 (n=2) pA/pF. The acquired data were curve-fitted, using a software (Fig. P version 6.0, BIOSOFT®), and showed the characteristics of Hill equation with a Hill number of 2.0, which is in the range of previously reported values of 1.6 (Gadsby *et al.*, 1988), 2.6 (Hilgemann, 1989) and 2.9 (Kimura *et al.*, 1987). And the Km value of the half saturation of fitted curve was 51.8±15.7 mM.

After confirming the Na^+-dependent out-

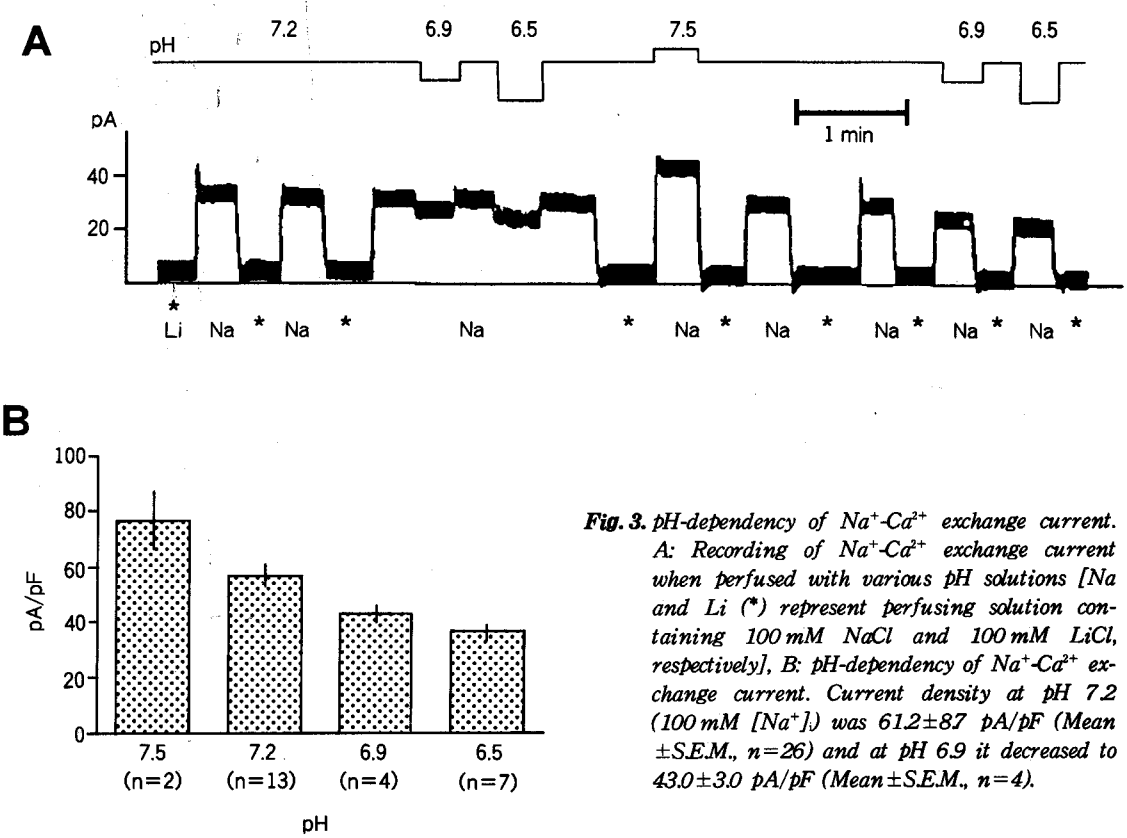

Fig. 3. *pH-dependency of Na^+-Ca^{2+} exchange current. A: Recording of Na^+-Ca^{2+} exchange current when perfused with various pH solutions [Na and Li (*) represent perfusing solution containing 100 mM NaCl and 100 mM LiCl, respectively], B: pH-dependency of Na^+-Ca^{2+} exchange current. Current density at pH 7.2 (100 mM $[Na^+]_i$) was 61.2±8.7 pA/pF (Mean ±S.E.M., n=26) and at pH 6.9 it decreased to 43.0±3.0 pA/pF (Mean±S.E.M., n=4).*

ward current as a current generated by Na^+-Ca^{2+} exchanger, the pH-dependency of the Na^+-Ca^{2+} exchange current was investigated. When pH in the perfusing solution was changed from 7.2 to 6.9, 6.5, and 7.5, the outward currents were decreased as the pH was lowered to 6.9 and 6.5, and increased as pH was raised to 7.5 (Fig. 3-A). The current densities were 76.9±10.3 pA/pF (n=2) at pH 7.5, 56.6±4.4 pA/pF (n=13) at pH 7.2, 42.9±3.0 pA/pF (n=4) at pH 6.9, 36.7±2.2 pA/pF (n=7) at pH 6.5, as shown in Fig. 3-B. The current density at pH 6.9 was 25% decremented compared to the current density at pH 7.2.

DISCUSSION

It is generally believed that cytosolic free calcium rises after periods of ischemia or hypoxia and that it is associated with postischemic cardiac damage (Renlund *et al.* 1984; Murphy *et al.* 1988; Tani 1990; Tani & Neely, 1990a, b; Weiss *et al.* 1990). However, the exact mechanism of calcium loading under these circumstances is not known. Haigney *et al.* (1992) suggested that the Na^+-Ca^{2+} exchange operating in a reverse mode might play a major role in calcium loading through exchanging extracellular Ca^{2+} for intracellular Na^+ that has accumulated during these periods.

In our previous study (Park *et al.* 1991; Kim *et al.* 1993), we measured intracellular Na^+ and Ca^{2+} activities of guinea pig papillary muscle during the high K^+ cardioplegia and the following recovery period using ion selective microelectrodes. The rate of increase in intracellular Ca^{2+} activity, when reperfused with

the Tyrode solution after ischemic cardiac arrest was dependent upon intracellular Na^+ activity established before the reperfusion in a fashion that the higher the intracellular Na^+ activity, the faster the rate of increase in intracellular Ca^{2+}. These results suggest that raised intracellular Na^+ activity is one of the risk factors of calcium paradox during reperfusion and the Na^+-Ca^{2+} exchange is one of the possible mechanisms for Ca^{2+} influx.

Na^+-Ca^{2+} exchange mainly operates in a forward mode (Na^+ influx coupled with Ca^{2+} efflux) during normal action potentials (Egan *et al.* 1989; Hilgemann, 1990). Under certain circumstances of sustained membrane depolarization to about -40 mV and intracellular Na^+ activities increased by high K^+ cardioplegia (Lazdunski *et al.* 1985; Pike *et al.* 1990; Tani & Neely, 1990a, b; Kim *et al.* 1993), the Na^+-Ca^{2+} exchanger could operate in a reverse mode (Haigney *et al.* 1992).

Ischemic cardiac arrest induces intracellular acidosis by anaerobic glycolysis (Allen & Orchard, 1987; Park *et al.* 1992) and this increase in H^+ activity is thought to affect the Na^+-Ca^{2+} exchanger (Mullins, 1977; Philipson *et al.* 1982; Haworth *et al.* 1987). Thus, the effects of intracellular acidosis on Na^+-Ca^{2+} exchanger during sustained ischemic cardiac arrest could be one of the main factors for enhancing intracellular accumulation of Na^+.

There have been some studies on the effect of pH on the Na^+-Ca^{2+} exchange using cardiac sarcolemmal vesicles (Philipson *et al.* 1982) and whole cell patch clamp with internal dialysis (Earm & Irisawa, 1986). However, those studies have some limitations in interpreting the results of these studies (Reeves & Philipson, 1989). It is generally believed that cardiac sarcolemmal vesicles have different membrane configuration from that of an intact sarcolemmal membrane (Reeves & Philipson, 1989). And internal organelles, present in whole cell patch experiments, may have secondary effects on the Na^+-Ca^{2+} exchanger. These experimental limitations can be partly overcome by the giant excised cardiac sarcolemmal membrane patch technique which utilizes a large patch of intact membrane free of internal organelles and was claimed to be very useful in studying the Na^+-Ca^{2+} exchanger (Hilgemann, 1989). Thus, to investigate the effect of intracellular acidosis, induced by high K^+ cardioplegia, on the Na^+-Ca^{2+} exchange activity is the specific aim of this study by measuring Na^+-dependent outward current generated by the isolated giant patch of cardiac sarcolemmal membrane.

Fig. 1 shows the currents measured through the giant sarcolemmal patch when the only currents generated by the Na^+-Ca^{2+} exchanger were left unblocked (see METHODS). These outward currents were manipulated to be generated by Na^+ efflux (moving into the pipette) coupled with Ca^{2+} influx (moving out of the pipette into the bathing solution) so that the measured currents represent the Na^+-Ca^{2+} exchange transport in reverse mode (as described above). An additional evidence was provided by partial blockage by known inhibitors of the Na^+-Ca^{2+} exchanger, Ni^{2+} and Co^{2+} (Kimura *et al.* 1987: Hilgemann, 1989). Na^+ dependence of these outward current showed typical characteristics of the "Hill equation", as shown in Fig. 2, with a Hill coefficient of 2.04 ± 0.74, which positions in the midst of previously reported values 1.6 (Gadsby *et al.* 1988) and 2.9 (Kimura *et al.* 1987), measured with whole cell patch configurations.

The current densities generated by the Na^+-Ca^{2+} exchanger was pH-dependent, in a way that the more acidic the cytosolic side is the smaller the current density. And acidification of the cytosolic side to pH 6.9 resulted in about a 25% decrement compared with pH 7.2. These data are largely compatible with the results of experiments using cardiac sarcolemmal vesicle (Philipson *et al.* 1982; Wakabayashi & Goshima, 1982; Slaughter *et al.* 1983) and whole cell clamp method (Earm & Irisawa 1986), and provide direct measurements of H^+ effect on Na^+-Ca^{2+} exchange transport.

Based on the results of Fig. 3 and our previous measurement of intracellular acidosis (Park *et al.* 1992) induced by high K^+ cardioplegia, down to pH 6.9, we can estimate that Na^+-Ca^{2+} exchanger activity could be reduced as much as 25% during the ischemic cardiac arrest. However, the Na^+-dependent outward

currents were measured at a membrane potential of 0 mV rather than −40 mV which can be induced by 25 mM K^+ cardioplegia. Thus, the current densities could be overestimated in this study. Our data suggests that an increased intracellular H^+, during cardioplegia, decreases the Na^+-Ca^{2+} exchange transport operating in a reverse mode which results in intracellular Na^+ accumulation. Further studies on the roles of other Na^+ transports, such as Na^+-H^+ exchange and Na^+ background current in intracellular Na^+ accumulation, will enrich the result of this study.

REFERENCES

Ahn DS, Lee SI, Kang DH: Mechanism of low-K^+ induced depolarization in mammalian cardiac muscle. *Yonsei Med J 28: 176-182, 1987*

Allen DG, Orchard CH: Myocardial contractile function during ischemia and hypoxia. *Circ Res 60: 153-167, 1987*

Earm YE, Irisawa H: Effects of pH on the Na^+-Ca^{2+} exchange current in single ventricular cells of the guinea pig. *Jpn Heart J 27 (suppl 1): 153-158, 1986*

Egan TM, Noble D, Noble SJ, Powell T, Spindler AJ, Twist VW: Sodium-Calcium exchange during the action potential in guinea-pig ventricular cells. *J Physiol 411: 639-661, 1989*

Fabiato A: Myoplasmic free calcium concentration reached during the twitch of an intact isolated cardiac cell and during calcium-induced release of calcium from the sarcoplasmic reticulum of a skinned cardiac cells from the adult rat or rabbit ventricle. *J Gen Physiol 78: 457-497, 1981*

Fiolet JWT, Baartscheer A, Schumacher CA, Coronel C, Ter Welle HF: The change of the free energy of ATP hydrolysis during global ischaemia and anoxia in the rat heart. Its possible role in the regulation of transsarcolemmal sodium and potassium gradients. *J Mol Cell Cardiol 16: 1023-1036, 1986*

Gadsby DC, Nakao M, Noda M, Shepherd N: [Na^+] dependence of outward Na/Ca exchange current in guinea-pig ventricular myocytes. *J Physiol 407: 135, 1988*

Grinwald PM: Calcium uptake during post-ischaemic reperfusion in the isolated rat heart. Influence of extracellular sodium. *J Mol Cell Cardiol 14: 359-365, 1982*

Hagiwara N, Irisawa H, Kasanuki H, Hosoda S: Background current in sinu-atrial node cells of the rabbit heart. *J Physiol 448: 53-72, 1992*

Haigney MCP, Miyata H, Lakatta EG, Michael DS, Silverman: Dependence of hypoxic cellular calcium loading on Na^+-Ca^{2+} exchange. *Circ Res 71: 547-557, 1992*

Haworth RA, Goknur AB, Hunter DG, Hegge JO & Berkoff HA: Inhibition of calcium influx in isolated adult rat heart cells by ATP depletion. *Circ Res 60: 586-594, 1987*

Hearse DJ: Reperfusion of the ischaemic myocardium. *J Mol Cell Cardiol 9: 605-616, 1977*

Hearse DJ, Humphrey SM, Bullock GR: The oxygen paradox and the calcium paradox, two facets of the same problem? *J Mol Cell Cardiol 10: 641-668, 1978*

Hilgemann DW: Giant excised cardiac sarcolemmal membrane patches: sodium and sodium-calcium exchange currents. *Pflugers Arch 415: 247-249, 1989*

Hilgemann DW: *"Best estimates" of physiological Na/Ca exchange function: Calcium conservation and the cardiac electrical cycle.* In Zipes DP, Jalife J, eds. *Cardiac electrophysiology.* Philadelphia, WB Saunders, *1990, pp 51-61*

Jennings RB, Scharper J, Hill ML, Steenbergen C Jr., Reimer KA: Effects of reperfusion late in the phase of reversible ischemic injury. *Circ Res 56: 262-278, 1985*

Kim MJ, Park SR, Suh CK: Effects of Na^+ and Ca^{2+} concentration in cardioplegic and reperfusion solutions on the intracellular Ca^{2+} of cardiac muscle cells. *Yonsei Med J 34: 133-144, 1993*

Kimura J, Miyamae S, Noma A: Identification of sodium-calcium exchange current in single ventricular cells of guinea-pig. *J Physiol 341: 325-339, 1987*

Kiyosue T, Spindler SJ, Noble SJ, Noble D: Background current $I_{b,Na}$ in guinea pig ventricular cells. *J Mol Cell Cardiol 24 (Sup 1): 269, 1992*

Lazdunski M, Frelin C, Vigne P: The sodium/hydrogen exchange system in cardiac cells: Its biological and pharmacological properties and its role in regulating internal concentrations of sodium and internal pH. *J Mol Cell Cardiol 17: 1029-1042, 1985*

Mullins LJ: A Mechanism for Na/Ca transport. *J Gen Physiol 70, 681-695, 1977*

Mullins LJ: Ion transport in heart. *Raven Press, New York, 1981, pp20-43*

Murphy JG, Smith TW, March JD: Mechanisms of reoxygenation-induced calcium overload in cultured chick embryo heart cells. *Am J Physiol 254: H1133-H1141, 1988*

Nayler WG: The role of calcium in ischemic myocardium. *Am J Pathol 102: 262-270, 1981*

Park SR, Kim MJ, Ahn DS, Suh CK: Effects of Na^+ and Ca^{2+} concentrations on the intracellular Ca^{2+} activity of cardiac muscle cells. *Biophysical J 59, 466a, 1991*

Park SR, Lee EH, Lee CJ, Suh CK: Effects of intracellular H^+ activity on the myocardial protection after high K^+ cardioplegia. *FASEB J 6, A443, 1992*

Philipson KD, Bersohn MM, Nishimoto AY: Effects of pH on Na^+-Ca^{2+} exchange in canine cardiac sarcolemmal vesicles. *Circ Res 50: 287-293, 1982*

Pike MM, Kitakaze M, Marban E: ^{23}Na measurements of intracellular sodium in intact ferret hearts during ischemia and reperfusion. *Am J Physiol 259: H1767-H1773, 1990*

Reeves JR, Philipson KD: Sodium-calcium exchange activity in plasma membrane vesicles. In Jeff T, Allen A, Noble D, Reuter H, eds. Sodium-Calcium exchange. *Oxford, Oxford University Press, 1989, pp 27-53*

Renlund DG, Gerstenblith G, Lakatta EG, Jacobus WE, Kallman CH, Weisfeldt ML: Perfusate sodium during ischemia modifies post-ischemic functional and metabolic recovery in the rabbit heart. *J Mol Cell Cardiol 16: 795- 801, 1984*

Shen AC & Jennings RB: Kinetics of calcium accumulation in acute ischemic myocardial injury. *Am J Pathol 67, 417-440, 1972*

Slaughter RS, Sutko JL, Reeves JP: Equilibrium calcium-calcium exchange in cardiac sarcolemmal vesicles. *J Biol Chem 258: 3183-3190, 1983*

Tani M: Mechanisms of Ca^{2+} overload in reperfused ischemic myocardium. *Annu Rev Physiol 52: 543-559, 1990*

Tani M, Neely JR: Role of intracellular Na^+ and Ca^{2+} overload and depressed recovery of ventricular function of reperfused ischemic rat hearts: Possible involvement of H^+-Ca^{2+} and Na^+-Ca^{2+} exchange. *Circ Res 65: 1045-1056, 1990a*

Tani M, Neely JR: Na^+ accumulation increases Ca^{2+} overload and impairs function in anoxic rat heart. *J Mol Cell Cardiol 22: 57-72, 1990b*

Wakabayashi S, Goshima K: Partial purification of Na^+-Ca^{2+} antiporter from plasma membrane of chick heart. *Biochim Biophys Acta 693: 125-133 1982*

Weiss RG, Lakatta EG, Gerstenblith G: Effects of amiloride on metabolism and contractility during reoxygenation in perfused rat hearts. *Circ Res 66: 1012-1022, 1990*