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Effects of Na^+ and Ca^{2+} Concentration in Cardioplegic and Reperfusion Solutions on the Intracellular Ca^{2+} of Cardiac Muscle Cells

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The removal of Ca^{2+} from the cardioplegic solutions could cause the danger of inducing a "calcium paradox" during reperfusion. Since intracellular Ca^{2+} activities are coupled to Na^+ activities via Na^+-Ca^{2+} exchange, an increase in intracellular Na^+ activities during the cardioplegia could cause an abrupt Ca^{2+} influx when reperfused. To study the effects of Na^+ and Ca^{2+} concentrations in cardioplegic solutions on intracellular Ca^{2+} activities during the cardioplegia and subsequent recovery period, the membrane potential and intracellular Na^+ and Ca^{2+} activities of guinea pig ventricular papillary were measured. 1) A cardioplegia with low Ca^{2+} cardioplegic solution significantly decreased the overshoot and duration of the first action potential after cardioplegia, but the changes in action potential configuration were minimized after a cardioplegia with Ca^{2+} concentration adjusted according to the Na^+-Ca^{2+} exchange mechanism. 2) Intracellular Na^+ activity was continuously decreased during the cardioplegia, and the intracellular Na^+ activity 20 minutes after cardioplegia was the highest with low Ca^{2+} cardioplegic solution. 3) Intracellular Na^+ and Ca^{2+} activities were continuously decreased during the cardioplegia with Ca^{2+} concentration adjusted according to the Na^+-Ca^{2+} exchange mechanism. 4) During a reperfusion of Tyrode solution after cardioplegia intracellular Na^+ and Ca^{2+} activities were increased. Intracellular Ca^{2+} activity was increased more rapidly than intracellular Na^+ activity. 5) The rate of increase in intracellular Ca^{2+} activity with reperfusion of Tyrode solution was dependent upon intracellular Na^+ activity during cardioplegia, in such a way that the higher the intracellular Na^+ activity was, the faster the intracellular Ca^{2+} activity increased. These data suggest that Na^+-Ca^{2+} exchange mechanism may play an important role in the regulation of intracellular Ca^{2+} activity during recovery after cardioplegia.

Key Words: Cardioplegia, guinea pig, ion selective microelectrode, intracellular Na^+ activity, intracellular Ca^{2+} activity, Na^+-Ca^{2+} exchange

High potassium cardioplegic solutions are used to arrest the heart during cardiac surgical procedures and to protect the heart tissue from the ischemic injury. The depolarization of the cell membrane induced with high K^+ along with hypothermia could reduce the energy expenditure of heart cells during the cardioplegia (Gay and Ebert, 1973; Roberts *et al.* 1980; McGoon, 1985; Prasad and Bharadwaj, 1987).

However, the depolarization of the heart cell could increase Ca^{2+} influx, and result in irreversible cardiac contracture due to intracellular Ca^{2+} loading (Mullins, 1981; Kimura *et al.* 1986; Mechmann and Pott, 1986; Kimura *et al.* 1987; Suh *et al.* 1988). To avoid intracellular Ca^{2+} overload, low Ca^{2+} solutions and/or

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Ca^{2+} antagonist containing cardioplegic solutions are frequently applied (Pinsky *et al.* 1981; Bourdillin and Poole-Wilson, 1982; Balderman *et al.* 1984; Hearse *et al.* 1984; Yamamoto *et al.* 1984; Hendriks *et al.* 1985; Tyres, 1988).

However, the removal of Ca^{2+} from the cardioplegic solutions could cause the danger of inducing a calcium paradox during the reperfusion, and subsequently irreversible reperfusion injury (Singal *et al.* 1986; Chapman and Tunstall, 1987; Sunnergren and Rovetto, 1987; Makino *et al.* 1988; Przyklenk and Kloner, 1989).

Intracellular Ca^{2+} activities are determined by the amount of Ca^{2+} influxed across the sarcolemmal membrane (via slow inward current and Na^+-Ca^{2+} exchange transport) and that of Ca^{2+} released from the SR (Winegrad, 1979; Sulakhe and St. Louis, 1980). As the depolarization of the heart cells are sustained, the amount of Ca^{2+} stored in the SR decreases (Allen *et al.* 1976; Bridge, 1986; Bers and Bridge, 1989) and Ca^{2+} channels get inactivated yielding a minimal Ca^{2+} influx (Noble, 1979; Hille, 1984). Thus Na^+-Ca^{2+} exchange transport plays an important role in regulating intracellular Ca^{2+} activities (Mullins, 1981; Sheu and Blaustein, 1986).

It has been known that Na^+ and Ca^{2+} have an important role in cardiac muscle contractility (Sulakhe and St. Louis, 1980; Mullins, 1981; Sheu and Blaustein, 1986). Ca^{2+} crosses the cell membrane into the sarcoplasm during the plateau of the action potential via the slow inward current, while the SR releases stored Ca^{2+} into the sarcoplasm. Increased sarcoplasmic Ca^{2+} then activates the contraction of the muscle cells, and following the contraction some of the Ca^{2+} is pumped into the SR and other Ca^{2+} is transported out of the cell, via Na^+-Ca^{2+} exchange transport.

Since intracellular Ca^{2+} activities are coupled to Na^+ activities via Na^+-Ca^{2+} exchange transport, an increase in intracellular Na^+ activities during the cardioplegia could cause an abrupt Ca^{2+} influx when reperfused. And it is necessary to consider the Na^+ concentration as well as Ca^{2+} for ideal cardioplegic solutions. Thus, in this study, the effects of Na^+ and Ca^{2+} concentrations in cardioplegic solutions on intracellular Ca^{2+} activities during the cardioplegia and subsequent recovery period were investigated, by measuring intracellular Na^+ and Ca^{2+} activities of guinea pig ventricular papillary.

METHODS

Guinea pigs of either sex ranging in size from 200 to 500 grams were sacrificed with an intraperitoneal injection of Na pentobarbital (50 mg/Kg) and heparin (2000 IU/Kg). The heart was rapidly excised and placed in oxygenated (100% O_2) Tyrode solution. Right ventricular papillary muscles were dissected, and then were transferred to a recording chamber which was continuously perfused with the oxygenated Tyrode solution. The Tyrode solution had the following composition in millimoles per liter (mM): NaCl 133.5; KCl 4.0; KH_2PO_4 0.3; $MgSO_4 7H_2O$ 1.2; $CaCl_2$ 1.8; HEPES

Table 1. Compositions of experimental solutions

	NaCl	TMA	$CaCl_2$	KCl	$MgCl_2$	NaH_2PO4	HEPES	Glucose
Tyrode	140	0	1.8	4	1	1	5	5.5
Cardioplegic solutions	140	0	1.8	25	1	1	5	5.5
	140	0	0.8*	25	1	1	5	5.5
	140	0	0	25	1	1	5	5.5
	126	14	0.6*	25	1	1	5	5.5
	98	42	0.28*	25	1	1	5	5.5

(mM)

*: Concentration of Ca^{2+} were adjusted according to the equilibrium of Na^+-Ca^{2+} exchange as described in DISCUSSION.

10; dextrose 10. All cardioplegic solutions had 25 mM K^+, and NaCl was isotonically replaced with tetramethylammonium (TMA) chloride to reduce the Na^+ concentration in cardioplegic solutions (Table 1). The flow rate of superfusion solutions was kept constant (about 5 ml/minute) in the way that the solution levels of solution containers were maintained constant with spring-aided small supports. The temperature in the recording chamber was maintained at 27.5±0.2℃. Tension was monitored by a Model 400A Force Transducer System (Cambridge Technology, Inc.) and displayed on a Philips PM 3305 Digital Storage Oscilloscope and on a Gould Brush 220 recorder along with the signal from the differential electrometer for the ion-selective electrode (ISE). Stimulating current provided by a Grass S11 stimulator was passed through a pair of platinum wires. Transmembrane action potentials were recorded between 3 M KCl-3% agar bridge in the bath and a standard microelectrode (RE) filled with 3 M KCl. The microelectrodes, pulled from microfiber capillary tubings (WPI, Inc.), had a typical resistance in the range of 5~20 megohms and tip diameters of less than 0.5 microns.

The measured transmembrane potentials were analyzed based on the non-parametric test (Wilcoxon-Mann-Whitney test) to evaluate differences between cardioplegic solutions.

Ca^{2+}-selective electrodes (CSE) and Na^+-selective electrodes (NSE) were manufactured as previously described (Suh *et al.* 1987; Park and Suh, 1991). The resin used for the Na^+-selective electrodes (NSE) was purchased from the Fluka Chemie AG (Fluka 71176). The resin used for the Ca^{2+}-selective electrodes was provided by Professor W. Simon. Polyvinylchloride (PVC), approximately 10% by weight, was added to the mixture for the improved stability of the CSE. Glass microelectrodes were pulled from borosilicate glass capillaries (WPI 1B200F6) and placed on the top of a bottle containing a small drop of pure dichlorodimethylsilane in an oven at 200℃ for 30 minutes. The silanized pipettes were bevelled with alumina powder and filled with reference solutions (100 mM NaCl for NSE; 1 mM $CaCl_2$ and 140 mM KCl for CSE). A column of exchanger resin (Na^+ or Ca^{2+} exchanger) was forced into the electrodes by means of a partial vacuum. The electrode was observed to be filled with resin to the tip length up to several 100 microns.

The e.m.f. (electromotive force) from the ISE was measured with an electrometer (AD515 operational amplifier, Analog Devices), and the membrane potential measured by the 3 M KCl filled microelectrode (RE) was electronically subtracted from the e.m.f. measured with the ISE. To improve a slow response time, the negative capacity compensation circuit was added to the head-stages of the amplifier. Na^+-selective electrodes and Ca^{2+}-selective electrodes were normally checked before and after the experiments. The Ca^{2+}-standard solutions for pCa 5, 6, 7, and 8 were made from a Ca^{2+} buffer containing EGTA (Suh, 1983) with an ionic background of 100 mM KCl. Na^+-selective electrodes were calibrated with mixed electrolyte solutions (NaCl 100+ KCl 40; NaCl 30+ KCl 110; NaCl 10+ KCl 130; NaCl 3, KCl 137; NaCl 1+ KCl 139; NaCl 0.3+ KCl 139.7; in mM). NSE had Nernstian responses in pure NaCl calibrating solutions, and in mixed NaCl-KCl solution began to deviate from Nernstian response at 30 mM NaCl plus 120 mM KCl (Park and Suh, 1991).

RESULTS

Effect of cardioplegic solutions on membrane potential

After ventricular papillary muscles were allowed to reach the steady-state contraction of 1 contraction per second (cps), 25 mM K^+ cardioplegic solutions, which have various concentrations of Na^+ and Ca^{2+}, were superfused for 20 minutes (cardioplegia). And then the tissue was superfused with normal Tyrode solution in order to facilitate recovery from the cardioplegia (recovery).

When the tissue was superfused with 100% Na^+/1.8 mM Ca^{2+} cardioplegic solution, the resting membrane potential was depolarized as much as 42.3 mV from −92.0±2.4 mV (n=18) and recovered to the value of the pre-cardioplegia (Fig. 1, Table 2). The cardioplegia with 98% Na^+/0.28 mM Ca^{2+} solution caused a significantly smaller magnitude of depo-

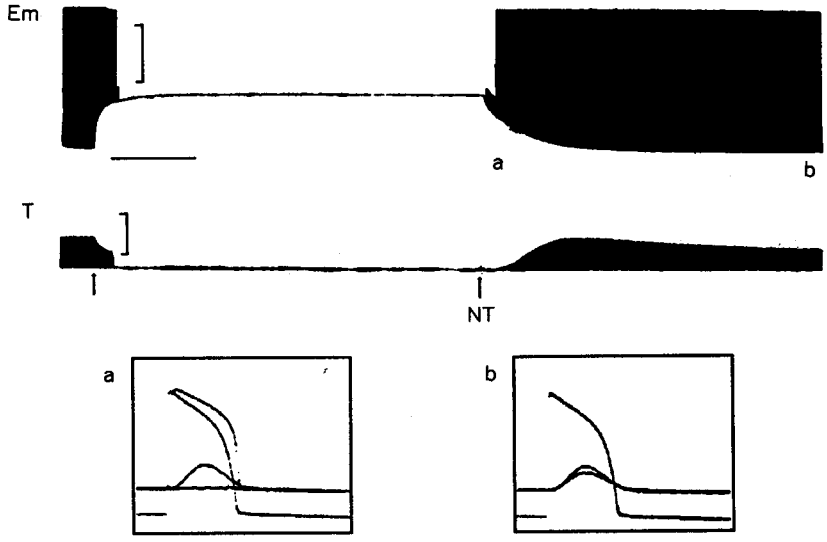


Fig. 1. *Effects of 25 mM K^+ cardioplegic solution on the action potential and contractile tension of ventricular papillary muscle. The tissue was arrrested for 20 minutes with 25 mM K^+ cardioplegic solutions (the first arrow) and recovered with the Tyrode solution (NT, the second arrow). Membrane potential (Em) and contractile tension (T) were measured at stimulus frequency of 1.0 cps. Action potential and tension of the first beating (a) and steady-state after 20 minute's recovery (b) after the cardioplegia were compared with those of steady-state beat before the arrest. Horizontal scale, 5 min.; Vertical scale, 40 mV for Em and 50 mg for T.*

Table 2. Effects of Na^+ and Ca^{2+} concentrations on the membrane potentials during and after the cardioplegia

Na^+ (mM)	Ca^{2+} (mM)	△Em (mV)		△Er (mV)	
140	1.8	43.3±2.2	(6)	+0.0±0.7	(5)
140	0.8	43.6±0.8	(5)	+0.5±1.0	(4)
140	0.1	44.2±1.5	(4)	−1.7±2.2	(4)
126	0.6	41.9±3.0	(4)	−1.4±1.4	(4)
98	0.28	39.6±2.5*	(4)	+1.3±2.2	(4)

Mean±S.D. (n)

*: $P<0.05$ (statistically significant compared to values of cardioplegic solutions containing 140 mM Na^+)

△Em: amplitude of depolarization during the cardioplegia

△Er: Changes in resting membrane potential after 20 minute's recovery from the cardioplegia relative to precadioplegic resting membrane potential

larization than other 100% Na^+ cardioplegic solutions tested (Table 2). However, the recovery was not affected with different composition of Na^+ and Ca^{2+}.

The action potentials of the first beats after cardioplegia, as the consequence of prolonged depolarization during cardioplegia were measured (Fig. 2 and Table 3). The configurations, overshoot and duration of the first action potential after cardioplegia were measured as an index of prolonged depolarization effects (Fig. 2 and Table 3). The magnitudes of overshoot and duration of the first action potential were decreased as Ca^{2+} concentration of cardioplegic solution was reduced. The first action potential after cardioplegia with 100% Na^+/0 Ca^{2+} solution had significantly reduced overshoot and shortened duration compared to ones after cardioplegia with other solutions (Fig. 2C and Table 3).

Based upon the equilibrium conditions of

Table 3. Effects of Na^+ and Ca^{2+} concentrations on the first action potential after cardioplegia

Na^+ (mM)	Ca^{2+}	Overshoot (mV)			Duration (msec)			
		AP_{SS}	AP_1	%	APD_{SS}	APD_1	%	
140	1.8	28.6±4.9	31.7±4.4	111.8± 7.7	228.6±29.1	370.0±38.8	163.8±23.9	(7)
140	0.8	32.2±1.8	22.8±5.5	70.3±13.5	250.0±12.3	242.0±80.1	96.9±31.3	(5)
140	0	31.3±1.2	11.0±7.9	34.7±24.2*	256.7±5.8	93.9±83.9	35.2±30.3#	(3)
126	0.6	31.3±3.0	28.8±6.5	91.7±16.7	207.5±5.8	255.0±67.6	124.4±35.5	(4)
98	0.28	29.6±1.7	17.2±6.9	58.8±25.7	224.0±36.5	214.0±41.0	107.2±30.3	(5)

Mean±S.D. (n)

*: $P<0.05$ (statistically significant compared to values of other cardioplegic solutions except solution containing 98 mM Na^+ and 0.28 mM Ca^{2+})

#: $P<0.05$ (statistically significant compared to values of cardioplegic solutions)

AP_{SS}: Overshoot of the steady-state action potential before the cardioplegia

AP_1: Overshoot of action potential of the first beat after the cardioplegia

APD_{SS}: Action potential duration of the steady-state action potential before the cardioplegia

APD1: Action potential duration of the first beat after the cardioplegia

Na^+-Ca^{2+} exchange during the 25 mM K^+ cardioplegia, the concentrations of Ca^{2+} in cardioplegic solution were estimated (see equation 2). After 100% Na^+/0.8 mM Ca^{2+} cardioplegic solution was superfused, the overshoot of the first action potential was decreased to 70% but the duration was almost the same as the precardioplegic action potentials (Fig. 2B, Table 3). When the Na^+ and Ca^{2+} concentration in cardioplegic solution were lowed to 90% (126 mM) and 0.6 mM respectively, the overshoot and duration of the first action potential were close to the precardioplegic action potential (Fig. 2D). A further decrease to 70% Na^+ (98 mM)/0.28 mM Ca^{2+} concentration in cardioplegic solution yielded a 60% decrease in overshoot and almost full recovery in duration of the first action potential (Fig. 2E). The contractile strength of the first beat was much stronger when the duration of the action potential was recovered close to the precardioplegic action potential (Fig. 2D & E).

Effects of cardioplegic solutions on intracellular Na^+ activity

The measured value of intracellular Na^+ activities (a_{Na}^i) was 7.9±0.32 mM (n=18)(in guinea pig ventricular papillary muscle). To investigate the effect of Ca^{2+} on a_{Na}^i, Ca^{2+} concentrations in cardioplegic solution were varied from 0.1 mM to 1.8 mM and the changes a_{Na}^i during cardioplegia, were observed from one tissue.

When the beat of the tissue was arrested with 1.8 mM Ca^{2+} solution, a_{Na}^i was 5.2 mM and decreased to 0.8 mM in 2 minute's cardioplegia, with a_{Na}^i of 4.4 mM. On the other hand, during the cardioplegia of 0.1 mM Ca^{2+} solution a_{Na}^i was decreased from 2.1 mM to 1.4 mM with a minimal change of 0.7 mM (Fig. 3). When another tissue was arrested with 3.6 mM Ca^{2+} cardioplegic solution, a_{Na}^i was decreased from 5.6 mM to 1.9 mM, compared to the decrease from 4.7 mM to 2.4 mM with 1.8 mM Ca^{2+} solution. These results imply that lower Ca^{2+} concentrations in 100% Na^+ cardioplegic solutions yield higher a_{Na}^i during cardioplegia.

Since a_{Na}^i were dependent upon Ca^{2+} concentration, the effects of cardioplegic s olutions whose Na^+ and Ca^{2+} concentrations were adjusted according the Na^+-Ca^{2+} exchange, were observed (Fig. 4). a_{Na}^i was decreased from 8.4 mM to 3.4 mM with 140 mM Na^+ / 0.8 mM Ca^{2+} solution, compared to a decrease from 6.7 mM to 4.2 mM with 140 mM Na^+/1.8 mM Ca^{2+} solution. And 98 mM Na^+/0.28 mM Ca^{2+} solution caused a_{Na}^i to decrease from 5.4 mM to 2.6 mM, showing that low Ca^{2+} concentration in cardioplegic solutions yielded lower a_{Na}^i, if Na^+ concentration was adjusted

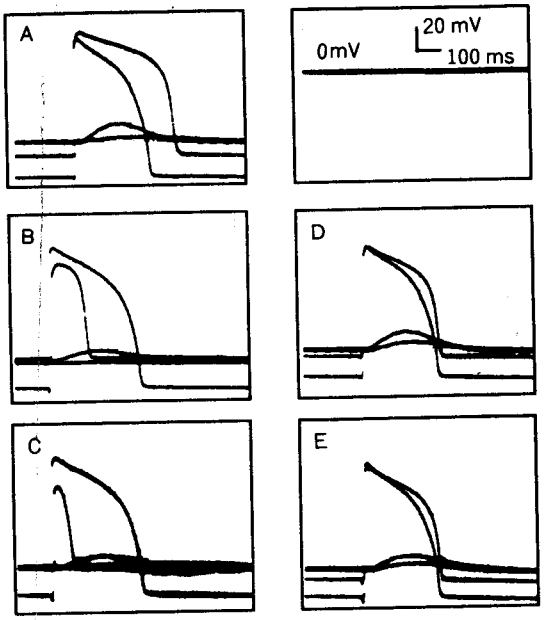
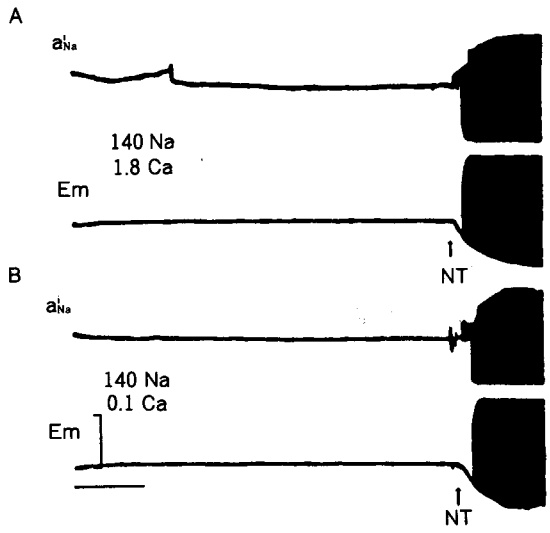


Fig. 2. *Effects of Na^+ and Ca^{2+} concentrations on the first action potential after cardioplegia. Action potential and tension of the first beating were compared with those of steady-state beat before the arrest. The tissue was arrested for 20 minutes with 25 mM K^+ cardioplegic solutions (A, 140 mM Na^+ and 1.8 mM Ca^{2+}; B, 140 mM Na^+ and 0.8 mM Ca^{2+}; C, 140 mM Na^+ and 0 mM Ca^{2+}; D, 128 mM Na^+ and 0.6 mM Ca^{2+}; E, 96 mM Na^+ and 0.28 mM Ca^{2+}) and recovered with the Tyrode solution (NT, arrow). Ca^{2+} concentrations in solutions D and E were adjusted according to the Na^+-Ca^{2+} exchange mechanism. Horizontal scale, 100 msec/div; Vertical scale, 20 mV/div.*



Na^+	Ca^{2+}	Duration of Cardioplegia (min)				
		stop	5	10	15	20
140	3.6	5.6	4.0	3.0	2.9	1.9
140	1.8	4.7	1.2	2.2	2.2	2.4
140	1.8	5.2	3.4	2.7	1.8	0.8
140	0.1	2.1	1.5	1.5	1.4	1.4

(mM)

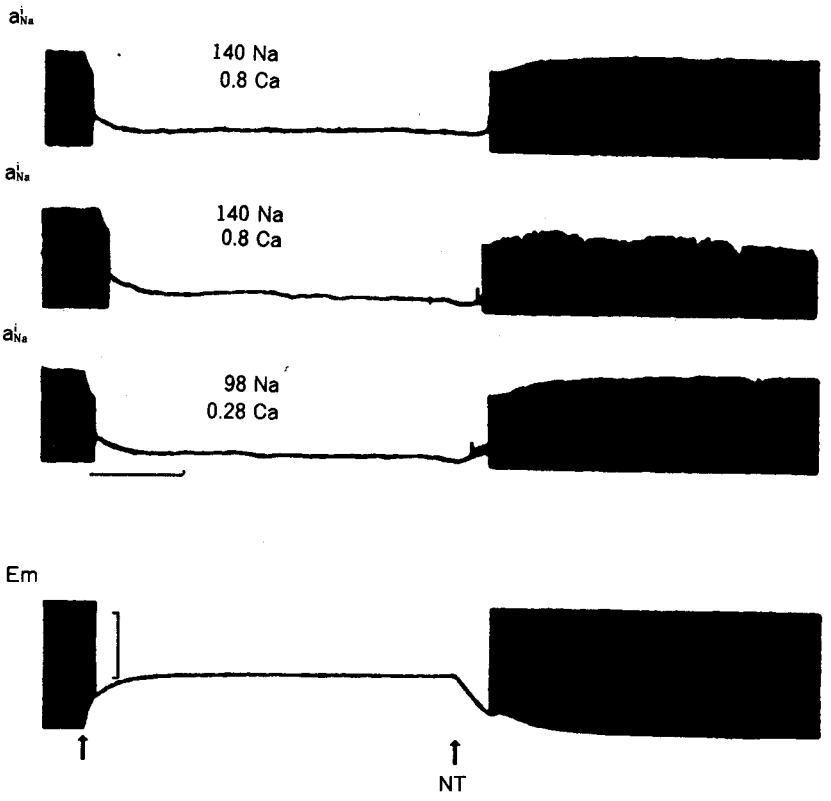
Fig. 3. *Effects of low Ca^{2+} concentrations in the cardioplegic solutions on the Na^+ activities during the cardioplegia. The tissue was arrested for 20 minutes with 25 mM K^+ cardioplegic solutions (A, 140 mM Na^+ and 1.8 mM Ca^{2+}; B, 140 mM Na^+ and 0.1 mM Ca^{2+}) and recovered with the Tyrode solution (NT, arrow). Horizontal scale, 5 min.; Vertical scale, 60 mV. Inset represents the intracellular Na^+ activities calculated from experimental data.*

accordingly.

Effect of cardioplegic solutions on intracellular Ca^{2+} activity

The measured value of intracellular Ca^{2+} activities (a^i_{Ca}) was 190±31 nM (n=29). To investigate the effect of Ca^{2+} concentration (1.8 mM and 0.1 mM) in cardioplegic solutions on a^i_{Ca}, the changes of a^i_{Ca} during cardioplegia were observed from one tissue (Fig. 5A). With 1.8 mM Ca^{2+} cardioplegic solution, a^i_{Ca} continuously decreased as much as 91.5% and rapidly increased when the tissue was reperfused with normal Tyrode solution. The rate of change in a^i_{Ca} during reperfusion was 121.2%/min.

With 0.1 mM Ca^{2+} cardioplegic solution, a^i_{Ca} decreased as much as 70.6% and increased as fast as 203.9%/min, when reperfused. These data implied that low Ca^{2+} concentration in cardioplegic solutions caused a rapid increase in a^i_{Ca} during reperfusion, even though a^i_{Ca} was



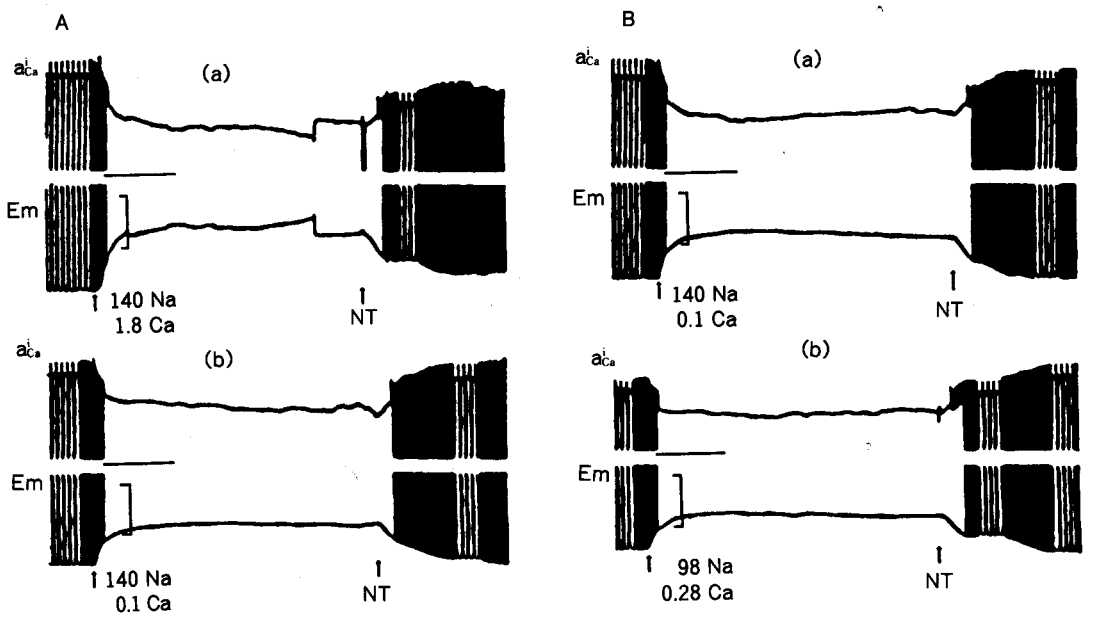
Na^+ (mM)	Ca^{2+} (mM)	Duration of Cardioplegia (min)				
		stop	5	10	15	20
140	1.8	6.7	3.8	4.2	4.4	4.2
140	0.8	8.4	3.8	4.2	3.4	3.4
9.8	0.28	5.6	2.6	3.0	3.2	2.6

(mM)

Fig. 4. *Effects of Na^+ and Ca^{2+} concentrations in the cardioplegic solutions on the intracellular Na^+ activities during the cardioplegia. The tissue was arrested for 20 minutes with 25 mM K^+ cardioplegic solutions (A, 140 mM Na^+ and 1.8 mM Ca^{2+}; B, 140 mM Na^+ and 0.1 mM Ca^{2+}) and recovered with the Tyrode solution (NT, arrow). Ca^{2+} concentrations in solutions B and D were adjusted according to the Na^+-Ca^{2+} exchange mechanism. Horizontal scale, 5 min.; Vertical scale, 60 mV. Inset represents the intracellular Na^+ activities calculated from experimental data.*

decreased to a less extent during cardioplegia.

Then the Na^+ and Ca^{2+} concentrations of cardioplegic solutions were adjusted according to Na^+-Ca^{2+} exchange (see the Discussion for details). During the cardioplegia with 100% Na^+/0.8 mM Ca^{2+} solution, a^i_{Ca} was decreased as much as 73% of a^i_{Ca} of the precardioplegia. With reperfusion, the rate of increase in a^i_{Ca}, before the first beat of postcardioplegia, was 159.3%/min (Fig. 5B). During the 70% Na^+/0.28 Ca^{2+} solution, a^i_{Ca} was decreased as much



Na^+ (mM)	Ca^{2+}	$\frac{\triangle[Ca^{2+}]_{20}}{[Ca^{2+}]_{SS}}$	$\frac{\triangle[Ca^{2+}]_R}{[Ca^{2+}]_{20}}$/ min
140	1.8	−91.5%	121.2%/min
140	0.8	−73.0%	159.3%/min
140	0.1	−70.6%	203.4%/min
98	0.28	−26.6%	159.3%/min

$[Ca^{2+}]_{SS}$: Intracellular Ca^{2+} activity at the beginning of cardioplegia
$[Ca^{2+}]_{20}$: Intracellular Ca^{2+} activity after 20 minutes of cardioplegia
$[Ca^{2+}]_R$: Intracellular Ca^{2+} activity at the first appearance of action potential during recovery
$\triangle[Ca^{2+}]_{20}$: $[Ca^{2+}]_{20}-[Ca^{2+}]_{SS}$
$\triangle[Ca^{2+}]_R$: $[Ca^{2+}]_R-[Ca^{2+}]_{20}$

Fig. 5. *Effects of Na^+ and Ca^{2+} concentrations in the cardioplegic solutions on the intracellular Ca^{2+} activities during the cardioplegia. The tissue was arrested for 20 minutes with 25 mM K^+ cardioplegic solutions and recovered with the Tyrode solution (NT, arrow). Concentrations of Na^+ and Ca^{2+} in cardioplegic solutions are as follows: A, 140 mM Na^+ and 1.8 mM Ca^{2+}; B, 140 mM Na^+ and 0.8 mM Ca^{2+}; C, 140 mM Na^+ and 0.1 mM Ca^{2+}; D, 98 mM Na^+ and 0.28 mM Ca^{2+}. Ca^{2+} concentrations in solutions B and D were adjusted according to the Na^+-Ca^{2+} exchange mechanism. Horizontal scale, 5 min.; Vertical scale, 60 mV. Inset represents the rates of changes in intracellular Ca^{2+} activities calculated from experimentral data.*

as 26.6%, and with reperfusion the rate of increase in a_{Ca}^i was 159.3%/min (Fig. 5B). The rate of increase in a_{Ca}^i was slower after the cardioplegia with adjusted solutions than with 100% Na^+/0.1 mM Ca^{2+} solution.

DISCUSSION

High K^+ cardioplegia could arrest cardiac beating by depolarizing myocytes during the

cardioplegia. However, the depolarization of heart cells could increase Ca^{2+} influx, and result in irreversible cardiac contracture due to intracellular Ca^{2+} loading (Hearse *et al.* 1984; Reimer and Jennings, 1986; Chapman and Tunstall, 1987; Suh *et al.* 1988). To avoid intracellular Ca^{2+} overload, low Ca^{2+} solutions and/or Ca^{2+} antagonist containing cardioplegic solutions are frequently applied (Pinsky *et al.* 1981; Bourdillin and Poole-Wilson, 1982; Balderman *et al.* 1984; Hearse *et al.* 1984; Yamamoto *et al.* 1984; Hendriks *et al.* 1985; Tyres, 1988). However, the removal of Ca^{2+} from the cardioplegic solutions could cause the danger of inducing a calcium paradox during the reperfusion, and subsequently irreversible reperfusion injury (Singal *et al.* 1986; Chapman and Tunstall, 1987; Sunnergren and Rovetto, 1987; Makino *et al.* 1988; Przyklenk and Kloner, 1989).

Intracellular Ca^{2+} activities are determined by the amount of Ca^{2+} influxed across the sarcolemmal membrane (via slow inward current and Na^+-Ca^{2+} exchange transport) and that of Ca^{2+} released from the SR (Winegrad, 1979; Sulakhe and St. Louis, 1980). As the depolarization of the heart cells are sustained, the amount of Ca^{2+} stored in the SR decreases (Allen *et al.* 1976; Bridge, 1986; Bers and Bridge, 1989) and Ca^{2+} channels get inactivated yielding a minimal Ca^{2+} influx (Noble, 1979; Hille, 1984). Thus Na^+-Ca^{2+} exchange transport plays an important role in regulating intracellular Ca^{2+} activities (Mullins, 1981; Sheu and Blaustein, 1986).

Thus the goal of this study is to maintain low intracellular Ca^{2+} activity during the sustained depolarization, namely cardioplegia, so that the cellular energy expenditure is minimized and equally importantly to keep intracellular Na^+ activity from being accumulated during the cardioplegia, which causes a rapid Ca^{2+} influx via Na^+-Ca^{2+} exchange during the reperfusion. In experiments, the changes in membrane potential and intracellular Na^+ and Ca^{2+} activities were measured to investigate the effects of Na^+ and Ca^{2+} concentrations in 25 mM K^+ cardioplegic solutions on intracellular Na^+ activity during the cardioplegia and consequent Ca^{2+} activity during the reperfusion. Although there are no experimental criteria on K^+ concentration in cardioplegic solutions, 25 mM of K^+ seems to restore cellular energy sources much better than lower concentration of K^+ (Tucker *et al.* 1970; Suh *et al.* 1988; Park *et al.* 1989).

The amplitude of the action potential is mainly determined by the Na^+ inward current, and Na^+ inactivation due to prolonged depolarization and/or a decrease in Na^+ gradient due to intracellular Na^+ accumulation are main causes of decrease in the Na^+ inward current. Conversely, the configurations of the first action potential after the cardioplegia could reveal the intracellular condition generated during the prolonged depolarization as shown in Fig. 2. Very short and small action potentials after cardioplegia with 0 Ca^{2+} solution, in Fig. 2C, may result from the decreased Na^+ driving forces due to the increase in intracellular Na^+ activity and relatively high degree of Na^+ inactivation. The latter part of probable causes described above could be possible since the membrane potential which elicited the first action potential was relatively higher with 0 Ca^{2+} solution than with other solutions. When the increase in intracellular Na^+ activity was minimized with adjustments of Ca^{2+} concentration in cardioplegic solutions (see below), the duration and overshoot of the first action potential was well recovered as shown in Fig. 2D & E.

Intracellular Na^+ activities during the cardioplegia are determined by Na^+ fluxes via the Na^+ pump, Na^+-Ca^{2+} exchange and Na^+ background current (Na^+ leak), depending on the degree of depolarization (Mullins, 1981; Gadsby *et al.* 1985; Ahn *et al.* 1987; Hagiwara *et al.* 1992; Kiyosue *et al.* 1992). In this study, 25 mM K^+ in cardioplegic solutions yielded membrane depolarization to about -40 mV during the cardiac arrest (Table 2). The activity of the Na^+ pump would be at a maximum, at a sustained membrane potential of -40 mV, yielding Na^+ efflux out of the cell (Eisner *et al.* 1981; Glitsch *et al.* 1981; Gadsby *et al.* 1985; Eisner, 1986). Na^+-Ca^{2+} exchange would also contribute to Na^+ efflux (Mullins, 1981; Sheu and Blaustein, 1986), resulting in a decrease in intracellular Na^+ activity during the sustained depolarization as shown in Fig. 3.

Since the mode of Na^+-Ca^{2+} exchange is also dependent upon Ca^{2+} (see equation 1), Ca^{2+} concentrations in cardioplegic solutions would

affect intracellular Na^+ activity. As shown in Fig. 3, the decline in Na^+ activities was augmented with higher concentration of Ca^{2+} in cardioplegic solutions. And a low Ca^{2+} concentration would increase the relative activity of Na^+ in cardioplegic solution, which possibly enhances Na^+ leak into the cells.

The Na^+-Ca^{2+} exchange process is a function of Na^+ and Ca^{2+} concentrations and the membrane potential across the cell membrane (Mullins, 1981). At membrane potential Vm, the ratio between intracellular Ca^{2+} and Na^+ concentration is described as follow

$$\frac{[Ca^{2+}]_i}{([Na^+]_i)^3} = \frac{[Ca^{2+}]_o}{([Na^+]_o)^3} \exp\left(\frac{V_m F}{RT}\right) \cdots\cdots\cdots (1)$$

In order to maintain this ratio of intracellular Ca^{2+} and Na^+ concentrations, at sustained depolarization ($\triangle$Vm) generated by the 25 mM K^+ cardioplegic solutions, Ca^{2+} and Na^+ concentrations in the cardioplegic solution must have a relationship with their respective concentrations in normal solution as described below,

$$\left|\frac{[Ca^{2+}]_o}{([Na^+]_o)^3}\right|_{CP} = \left|\frac{[Ca^{2+}]_o}{([Na^+]_o)^3}\right|_{NT} \exp\left(\frac{VmF}{RT}\right) \cdots\cdots\cdots (2)$$

where CP and N represent cardioplegic and normal solutions respectively.

Thus, restraining intracellular Na^+ activity accumulation would require a low Na^+ concentration in cardioplegic solutions, and equally importantly Ca^{2+} concentration adjusted according to the equation (2) to avoid intracellular Ca^{2+} overload. The results of Fig. 4 present the experimental support for the theoretical estimation from equation (2). Although there have been reports suggesting intracellular Ca^{2+} increase during the cardioplegia (Hearse *et al.* 1984; Hendriks *et al.* 1985), intracellular Ca^{2+} activities continuously declined during the sustained depolarization and abruptly increased as the ventricular papillary was reperfused with normal Tyrode solution (Suh *et al.* 1988; Fig. 5). These results imply that intracellular Na^+ accumulates and intracellular Ca^{2+} activity decreases during the cardioplegia and provides a driving force for Ca^{2+} influx via Na^+-Ca^{2+} as exchange is restored. The results of Fig 3 and 4 show that the lower the Ca^{2+} concentration is in the cardioplegic solution, the lower the intracellular Ca^{2+} activity is and the higher the intracellular Na^+ activity is during the sustained depolarization. These phenomena can be explained as follows. During the sustained depolarization, Na^+ influx via Na^+ background current increases, causing intracellular Na^+ accumulation and a subsequent decrease in Ca^{2+} efflux via decremented activity of the Na^+-Ca^{2+} exchange. (The change in Na^+ background currents is not covered in this study but will be published in next paper. Park and Suh, 1993).

Decreased intracellular Ca^{2+} activities may provide a larger Ca^{2+} concentration gradient during the early phase of reperfusion, which is not clear in this study. However, it seems that intracellular Na^+ accumulation plays a main role in Ca^{2+} influx via Na^+-Ca^{2+} exchange as shown in Fig. 5. The main benefit of this study is that maintenance of low intracellular Na^+ activity during the cardioplegia can prevent the reperfusion injury due to calcium paradox, by way of adjusting Na^+ and Ca^{2+} concentration in cardioplegic solutions as recommended in equation (2).

After the heart beating is restarted, intracellular Ca^{2+} activity is determined by Ca^{2+} influx via Ca^{2+} inward current during the plateau of the action potential, Ca^{2+} flux via Na^+-Ca^{2+} exchange and Ca^{2+} released from the SR. Since intracellular Na^+ and Ca^{2+} could be decrease during the sustained depolarization, the possibility that Ca^{2+} enters via Na^+-Ca^{2+} exchange during the action potential draws much attention for future study, event though there have been several negative reports on this matter (Hilgemann, 1988; Egan *et al.* 1989).

Intracellular H^+ activity is also one of the important factors for myocardial protection because a change in Na^+ activity surely influences H^+ activity along with intracellular metabolic acidosis. Thus, the study on the role of Na^+-H^+ exchange in intracellular Na^+ modulation during the cardioplegia via Na^+-background current and Na^+-Ca^{2+} exchange will surely enhance the beneficial result of this study.

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