Transpl Int (2001) 14: 72–79

ORIGINAL ARTICLE

Makoto Sunamori
Masato Shimizu
Noriyuki Tabuchi
Hirokuni Arai
Hiroyuki Tanaka

The use of a nondepolarizing cardioplegic solution for cardiac preservation has a beneficial effect on the left ventricular diastolic function

Received: 14 October 1999
Revised: 30 October 2000
Accepted: 21 December 2000

M. Sunamori (✉) · M. Shimizu
N. Tabuchi · H. Arai · H. Tanaka
Department of Thoracic-Cardiovascular Surgery, Tokyo Medical and Dental University, 1-5-45, Yushima, Bunkyo-ku, Tokyo, 113–8519 Japan
e-mail: sunamori.tsrg@med.tmd.ac.jp
Tel.: + 81-3 5803-5267
Fax: + 81-3 5803-5267

Abstract We have developed a nondepolarizing solution (NDS) that retards myocardial calcium accumulation during cardioplegia. This study compares 1) the membrane resting potential (Em) in Purkinje fibers during cardioplegia induced by NDS or University of Wisconsin solution (UW) at normothermia and hypothermia for 6 h, 2) left ventricular (LV) diastolic function of isolated canine hearts preserved with NDS or UW for 6- and 12 h in hypothermia to elucidate the relationship between diastolic function and myocyte physiology ($n = 8$, each group), and 3) the effect of Non-depolarizing solution (NDS) compared with Bretschneider's HTK solution on LV diastolic function in isolated rabbit hearts using the Langendorff model in normothermia ($n = 10$, each group). The membrane resting potential (Em) was as follows: NDS in normothermia, –71 mV (2 min), –65 mV (30 min), and –52 mV (60 min); NDS in hypothermia, –40 mV (1 h) and –32 mV (6 h), while UW in hypothermia 0 mV (6 h). Myocardial calcium accumulation during reperfusion in the NDS groups was minimal and significantly lower than in the UW groups after the 6- and 12 h preservations. Postreperfusion myocardial cyclic adenosin monophosphate (cAMP) and adenosin triphosphate (ATP) concentrations in the NDS groups were closer to normal than in the UW groups after the 6- and 12 h preservations. The postreperfusion myocardial Ca concentration correlated with the cAMP ($r = -0.68$, $n = 25$, $P = 0003$) and cyclic guanosine monophosphate (cGMP) concentrations ($r = -0.69$, $n = 25$, $P = 0.003$). The left ventricular end-diastolic pressure (LVEDP) after reperfusion correlated with myocardial ATP ($r = -0.65$, $n = 25$, $P = 0.003$) and Ca concentrations ($r = -0.68$, $n = 25$, $P = 0005$). However, the parameter indicating LV elasticity (max LV –dp/dt) correlated with neither the Ca or ATP concentration following reperfusion. NDS prevented stiffness (increased LVEDP) better than HTK during normethermic cardioplegia for 30 min. These results *in vitro* suggest that NDS prevents myocardial Ca accumulation, depletion of ATP and cAMP, and preserves LV diastolic function, particularly stiffness after reperfusion, for up to 12 h. Furthermore, the mycoardial Ca concentration is inversely correlated with the cAMP and cGMP concentrations.

Keywords Cardioplegia · nondepolarization · diastolic function of the left ventricle · membrane resting potential

Abbrevations *AMP* Action membrane potential · *ATP* Adenson triphosphate · *cAMP* Cyclic adenosine monophosphate · *cGMP* Cyclic guanosine monophosphate · *Em* Membrane resting potential · *LV* Left ventricular · *LVEDP* Left ventricular end-diastolic pressure · *NDS* Non-depolarizing solution · *PKA* Protein kinase A

Introduction

Left ventricular dysfunction, particularly, diastolic dysfunction, is a major problem following prolonged ischemia. It can occur during cardiac preservation. Hypothermic cardioplegia is widely used for myocardial preservation but causes enzyme dysfunction [11], decreased membrane stability [6, 12], and calcium sequestration [2], which induces other ion fluxes secondarily. Our results [14], and those of other investigators [21], have shown that myocardial calcium accumulation not only increases left ventricular wall stiffness, but also depletes myocardial ATP, which in turn impedes ventricular relaxation. These alterations lead to ventricular dysfunction, depletion of myocardial high energy compounds, and myocardial necrosis. Accumulated evidence suggests that ischemia-reperfusion injures the sarcolemma and alters receptors on the membrane through enzymes bound to the sarcolemma. These signal transduction pathways play specific roles in ion movement, inflammation, biosynthesis of nucleotides, and contraction of myofibrils.

Currently used myocardial preservation solutions are classified as intracellular, extracellular and intermediate type. A typical intracellular solution is University of Wisconsin (UW) solution, and an extracellular solution is the St. Thomas solution or Bretschneider's HTK solution. These solutions induce cardiac arrest by depolarizing the heart. The degree of depolarization depends on the concentrations of electrolytes, primarily potassium and secondarily sodium. It is well known that both hypothermia and ischemia elevate the resting membrane potential toward zero, to produce instability of sarcolemma. We found that nondepolarizing solutions protect the membrane during ischemia-reperfusion and enhance left ventricular recovery [23]. Furthermore, the nondepolarized state permits up to 12 h of preservtion with satisfactory return of left ventricular systolic function in a canine model [24]. Complete polarization can be obtained only by tetrodotoxin, a sodium channel blocker to the preservation solution [22, 16]. However, the side effects of tetrodotoxin prohibit its use clinically, and no other sodium channel blocker is available. Consequently there is no way to maintain complete polarization in practice. From a theoretical perspective, the nondepolarized (polarized) state is resistant to the electrophysiological changes that are secondary to ischemia and reperfusion. We hypothesise the following: 1) the nondepolarized state prevents calcium overload, and subsequently maintains higher myocardial ATP concentrations following ischemia-reperfusion, which thereby preserves left ventricular diastolic function, and 2) in normothermia, since the deleterious effect of hypothermia are eliminated, the protective effect of the nondepolarized state on diastolic function may be greater than that of the depolarized state.

This study tests our hypotheses using two experiments. First, isolated canine hearts were preserved hypothermically using UW solution or nondepolarizing solution to study biochemical changes, and changes in left ventricular diastolic function; Second, under normothermia, changes in left ventricular end-diastolic pressure in isolated rabbit hearts induced by HTK solution or nondepolarizing solution were compared.

Materials and methods

Study of membrane resting potential in the Pukinje fibers of guinea pig

The resting membrane potential (Em) was measured following cardioplegia for 6 h at 5 °C and for 1 h at 36 °C for 1 h cardioplegia using the left ventricular papillary muscles of guinea pig hearts. Our cardioplegic solution consisted of NaCl 60 mmol/l, KCl 0 mmol, $CaCl_2$ 1 mmol, Mg-l-aspartate 8 mmol, glucose 245 mmol, mannitol 50 mmol, betamethasone 250 mg, lidocaine hydrochloride 1 mmol and sodium bicarbonate 10 mmol/l in distilled water. The pH was 7.5, and the osmolarity was 450 mOsm/l.

Preparation

Each guinea pig (400–500 g) was anesthetized with pentobarbital (intravenous, 30 mg/kg). The heart was immediately excised, and the papillary muscle and Purkinje fibers were isolated from the left ventricle and transferred to oxygenated Tyrode solution maintained at 36 °C. The composition of the Tyrode solution was: NaCl 130 mmol, KCl 5 mmol, $CaCl_2$ 2 mmol, $MgCl_2$ 1 mmol, glucose 10 mmol, Na-HEPES 10 mmol. The pH was 7.4 at 36 °C.

Microelectrodes

The membrane potential was measured with conventional 3 M KCl-filled glass micro-electrodes that had a resistance of $4–10 \times 10^6 \Omega$. The Em was corrected for a change in the liquid junction potential of the 3 M KCl-agar electrode in different solutions. Conventional electrodes were connected to high impedance input probes of a dual/differential electrometer with Ag/AgCl pellet microelectrode holders. The bath was coupled to ground via a 3 M KCl-agar bridge and a calomel electrode. The electrometer outputs were displayed on a pen recorder.

Experimental procedure

Purkinje fibers were mounted in a small experimental bath and continuously superfused at rates of 2–3 ml/min. The superfusing medium could be changed rapidly, with a complete exchange accomplished within 60 s. Purkinje fibers were equilibrated at least 1 h in the Tyrode solution at 36 °C. Acceptable electrical coupling of the impaled cells was assured by measuring action potentials induced by electrical stimuli. The Em and action membrane potential (AMP) were recorded in Tyrode solution at 36 °C as control. Then the superfusing medium was changed to cardioplegic solution, and the Em was measured and recorded with a pen-recorder (Nihon Kohden, Tokyo). The interval until electrical arrest occurred was noted. After this, the bath medium was changed back

to oxygenated Tyrode solution at 36 °C, and the Em and AMP were recorded continuously as soon as the bath had been replaced with the Tyrode solution.

Experimental group

Group A-I ($n = 7$): arrest was maintained for 1 h at 36 °C with nondepolarizing cardioplegic solution, group A-II ($n = 7$): arrest was maintained for 6 h at 5 °C using UW solution (group IIa) or nondepolarizing cardioplegicc solution (group IIb).

Experimental study on left ventricular diastolic function of the reperfused donor heart

Thirty-two mongrel dogs weighing 10–21 kg were anesthetized with intravenous pentobarbital 30 mg/kg, and maintained by mechanical ventilation.

Procurement of the heart

A median sternotomy was performed, and the superior and inferior vena cavae were isolated with 2–0 silk ligatures, both proximally and distally. The azygous vein was doubly ligated and divided. Both common carotid arteries, the left subclavian artery, and the descending aorta were isolated with 2–0 silk, proximally and distally, as were the hila of both lungs. A 10 F arterial cannula was inserted from the proximal right subclavian artery, and a 24 F venous cannula was placed in the right ventricle through the right atrial appendage. Approximately 500 ml of blood was withdrawn from the venous cannula, heparinized, and saved for transfusion during reperfusion. The previously isolated arteries and pulmonary hila were ligated after ventilation was terminated. Immediately after aortic occlusion, cardioplegia was induced by the infusion of cold (5 °C) cardioplegic solution (15 ml/kg) via the arterial cannula with infusion pressure of 50 cm in water. The superior and inferior vena cavae were ligated and divided, the the heart was removed.

Preservation of the heart

The hearts were divided into four groups depending on the type of cardioplegic solution [University of Wisconsin (UW, ViaSpan, E. I. du Pont de Nemours, Wilmington, Del.) or nondepolarizing solution (NDS)] and the duration of preservation [6 or 12 h]. Hearts were immersed in the same cardioplegic solution at 5 °C as used to induce cardioplegia. NDS was composed of Na^+ 70 mmol, Mg^{2+} 8 mmol, Ca^{2+} 1 mmol, glucose 245 mmol, mannitol 50 mmol, lidocaine hydrochloride 1 mmol/l and betamethasone 250 mg/l, at 450 mOsm/l buffered with sodium bicarbonate to pH 7.5. Cardioplegic solution 3 ml/kg was injected hourly at an infusion pressure of 50 cm in water in all groups during preservation.

One hour prior to reperfusion, a latex balloon was placed in the left ventricle and secured with a holding apparatus sutured in the mitral position. The balloon was connected to a transducer (Statham P23DB, Statham Instruments, Los Angeles, Calif.), and the left ventricular pressure during reperfusion was recorded with a polygraph (Nihon Kohden, Tokyo). Special care was taken to avoid mechanically induced aortic regurgitation. Thirty minutes prior to reperfusion, the hearts were returned to room temperature by discontinuing immersion in cold cardioplegic solution.

Reperfusion

A second dog was anesthetized, ventilated, and maintained hemodynamically by the infusion of lactated Ringer's solution. Both carotid arteries were cannulated with a 10 F catheter and connected to the arterial cannula placed in the preserved heart. A second pressure transducer and magnetic flow meter (Nihon Kohden) were connected to the circuit to measure perfusion pressure and flow. Blood from the cannula in the right ventricle and from the left ventricle was collected in the reservoir and infused back into the supporting dog by a pump; a heat exchanger maintained normothermia. Reperfusion was continued for 2 h. Defibrillation was performed whenever ventricular fibrillation developed during the early phase of reperfusion. After 5 min of reperfusion, all dogs were paced at 130 beats per minute. No cardiotonic drug was administered to any of the dogs.

At the end of cardiac arrest and during reperfusion, a biopsy specimen of the left ventricular subendocardium was obtained while the heart was beating. Myocardial concentrations of adenosine triphosphate (ATP) were measured using previously described methods [43], calcium concentrations measured with atomic absorption spectrophotometry, and cyclic adenosine monophosphate (cAMP) and guanosine monophosphate (cGMP) concentrations were measured with radioimmunoassay.

Experimental groups

The hearts were divided into four groups of eight hearts each depending on the type of cardioplegic solution and the duration of preservation. Group UW6, preservation in UW solution for 6 h; group UW12, preservation in UW solution for 12 h; group ND6, preservation in NDS for 6 h; group ND12, preservation in NDS for 12 h.

Effect of Em induced by cardioplegia on post-ischemic left ventricular diastolic function in normothermia

Isolated hearts prepared from Japanese white rabbits (2.5–3.5 kg) were used in the Langendorff perfusion model. Animals received care according to the "Principles of Laboratory Animal Care" formulated by the National Society for Medical Research and the "Guide for the Care and Use of Laboratory Animals" prepared by the National Academy of Sciences. Hearts were arrested by infusion of Bretschneider's HTK solution (Custodiol, group C1, $n = 10$) or nondepolarizing solution (NDS, group C2, $n = 10$). Cardioplegic arrest was maintained for 30 min followed by 30 min of reperfusion in normothermia. During reperfusion, the left ventricular diastolic pressure was measured using a latex balloon placed in the LV cavity and connected to a pressure-transducer and a polygraph.

Statistical analysis

Data are expressed as mean ± SEM. Data within a group were analyzed by Student's paired t test and between groups by the Bonferroni-Dunn test using analysis of variance (ANOVA). Incidence of ventricular fibrillation was analyzed by Chi-square test. Regression analysis was performed with 95 % confidence intervals. P values < 0.05 were considered statistically significant.

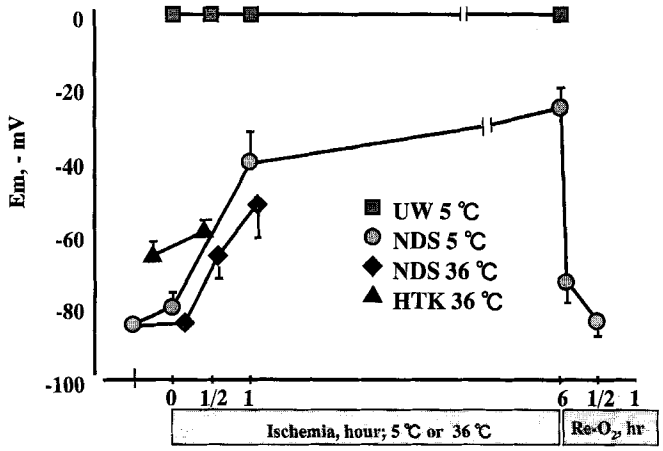


Fig. 1 Membrane resting potential (*Em*) induced by University of Wisconsin (*UW*), Bretschneider's *HTK* and Nondepolarizing cardioplegic solution (*NDS*) at 5 °C and 36 °C. The *Em* was measured in guinea pig Purkinje fibers by microelectrode. Data are expressed as mean ± SEM. $n = 6$ in each group. Re: reperfusion. Data on Bretschneider's HTK solution was cited from reference 28

Results

Membrane resting potential

Changing the solution from standard Tyrode solution to NDS caused electrical arrest. The Em after 3 min was −70.6 ± 4.7 at 36 °C and −79.7 ± 11.9 mV at 5 °C. These values were comparable to controls. Em remained unchanged during the first 30 min of ischemia, but during the next 30 min, the Em slowly rose to −52.0 ± 11.9 at 36 °C (group I) and −38.6 ± 8.0 mV at 5 °C (group IIa, $P < 0.05$). During cardioplegia, Purkinje fibers did not respond to electrical stimuli. The time to arrest was 160.2 ± 63.2 s at 36 °C and 41.7 ± 36.5 s at 5 °C ($P < 0.05$). With re-introduction of normal Tyrode solution (reperfusion, 30 min), the Em recovered to the control level in both groups. However, the membrane action potential in group II (5 °C) remained smaller than in group I (36 °C) ($P < 0.05$) (Fig. 1). On the other hand, Em induced by UW solution was 0 mV at 5 °C throughout the experiment.

Left ventricular diastolic function and myocardial biochemistry after hypothermic preservation

The incidence of ventricular fibrillation was significantly different between NDS and UW groups: 0 (ND6) vs 87.5 % (UW6); 0 (ND12) vs 100 % (UW12). Myocardial Ca, cAMP, cGMP, and ATP concentrations are shown in the Table. The myocardial calcium concentration after preservation decreased in group UW-6 and 12, but was unchanged in groups NDS-6 and 12. The myocardial Ca concentration after reperfusion increased in both the UW- and NDS-treated groups. However, calcium overload in groups NDS-6 and 12 was less than in the UW groups. The myocardial ATP concentration after preservation was the same in all groups, and the myocardial ATP concentration after reperfusion was unchanged in group NDS-6 and 12, but decreased in groups UW-6 and 12. The myocardial cAMP concentra-

Table 1 Left ventricular diastolic function and biochemical parameters in hearts preserved with nondepolarizing solution and University of Wisconsin solution for 6 and 12 h at 5 °C. Data are presented as mean ± SEM, $n = 8$ for all groups. *B* Baseline without ischemia, *P* end of preservation, *R* end of reperfusion, *LVEDP* left ventricular end-diastolic pressure, *Ca* calcium, *cAMP* cyclic adenosine monophosphate, *cGMP* cyclic guanosine monophosphate, *ATP* adenosine tri-phosphate, *LV* diastolic function is shown by values measured using a balloon inflated with 10 ml of saline. * Indicates $P < 0.05$ relative to UW

Experimental group		Non-depolarizing solution		University-Wisconsin solution	
Duration of preservation, h		6	12	6	12
Ca, tissue, ug/g,	B	42.9 ± 3.1	42.9 ± 3.1	42.9 ± 3.1	42.9 ± 3.1
	P	40.4 ± 3.5*	42.8 ± 2.4*	21.0 ± 1.1	16.4 ± 1.9
	R	66.1 ± 3.2*	75.5 ± 6.2*	144.7 ± 17.7	135.8 ± 17.0
cAMP, tissue, pg/g,	B	586 ± 87	586 ± 87	586 ± 87	586 ± 87
	P	2560 ± 208	2270 ± 136	2292 ± 226	2435 ± 560
	R	908 ± 117*	384 ± 49*	231 ± 42	150 ± 63
cGMP, tissue, pg/g,	B	10.9 ± 3.3	10.9 ± 3.3	10.9 ± 3.3	10.9 ± 3.3
	P	12.3 ± 1.6	22.6 ± 3.3	15.8 ± 2.7	23.4 ± 3.4
	R	10.7 ± 0.7	10.4 ± 1.4	7.5 ± 1.1	7.2 ± 0.6
ATP, tissue, umol/g, dry	B	22.35 ± 0.87	22.35 ± 0.87	22.35 ± 0.87	22.35 ± 0.87
	P	28.7 ± 3.9	31.2 ± 3.4	31.3 ± 0.4	34.0 ± 8.6
	R	32.6 ± 4.2*	27.3 ± 5.3*	11.6 ± 3.3	7.2 ± 3.4
LVEDP, mm Hg,	R	7.9 ± 5.5*	38.0 ± 11.1*	49.4 ± 16.1	102.5 ± 26.6
LV-dp/dt, mm Hg/sec,	R	735 ± 78	570 ± 90*	788 ± 200	150 ± 79

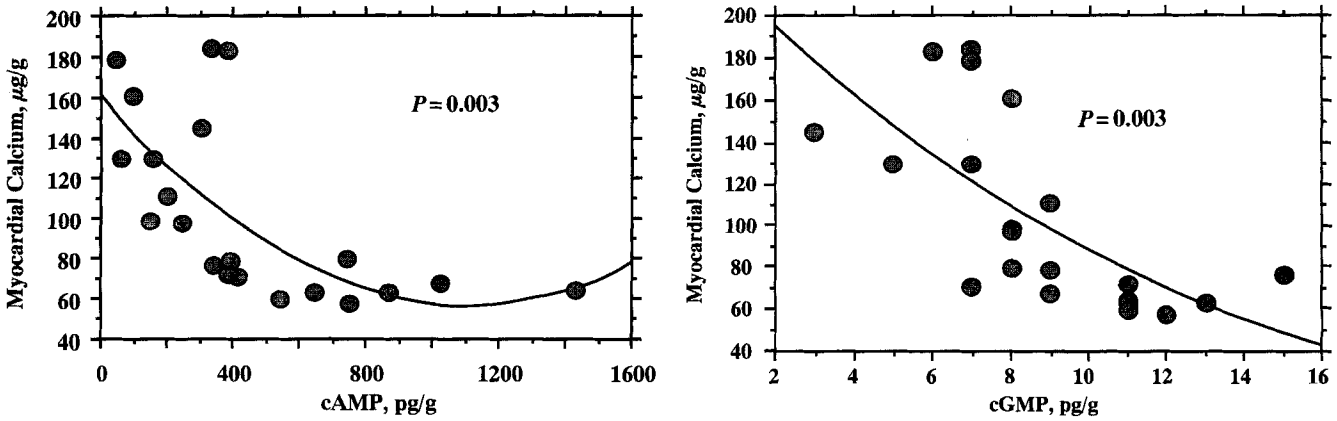


Fig. 2 Correlation between concentrations of myocardial Ca and high energy phosphate compounds following reperfusion after cardioplegia. *Left panel* Regression between myocardial Ca and cAMP after 2 h reperfusion following 6- or 12 h preservation of canine hearts. $r = -0.68$, $n = 21$, $P = 0.003$. *cAMP* Cyclic adenosine monophosphate. *Right panel* Regression between myocardial calcium and cGMP after 2 h reperfusion following 6- or 12 h reperfusion of canine hearts. $r = -0.69$, $n = 21$, $P = 0.003$. *cGMP* Cyclic guanosine monophosphate

tion after reperfusion was higher in groups NDS-6 and 12 than in groups UW-6 and 12. (Table 1)

Left ventricular end-diastolic pressure (LVEDP) after reperfusion was normal in group NDS-6 and was slightly elevated in group NDS-12. However, the LVEDP after reperfusion in groups UW-6 and 12 was markedly elevated and higher than that of the NDS group. Left ventricular negative dp/dt (LV-dp/dt) after reperfusion was depressed in all groups. However, LV-dp/dt after reperfusion was better in group NDS-12 than in group UW-12. (Table 1)

Regression analysis showed the following relationships: myocardial Ca concentration correlated with cAMP concentration ($r = -0.68$, $n = 21$, $P = 0.003$, Fig. 2 left) and with the cGMP concentration ($r = -0.69$, $n = 21$, $P = 0.003$, Fig. 2, right). The LVEDP correlated with the myocrdial ATP concentration ($r = -0.65$, $n = 23$, $P = 0.004$, Fig. 3, left) and myocardial Ca concentration ($r = -0.69$, $n = 23$, $P = 0.005$, Fig. 3, right). The LV-dp/dt did not correlate with the myocardial ATP content.

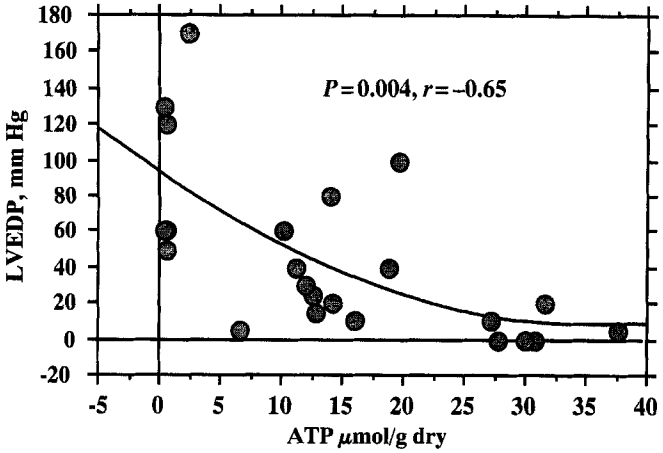
Changes in the LVEDP after normothermic cardioplegia (30 min) using HTK-solution or NDS

The LVEDP after reperfusion was significantly lower for LV volumes ranging from 0.1 to 2.0 ml in the NDS group than that of the HTK group (Fig. 4).

Discussion

The effect of cardioplegic solution on the resting membrane potential

NDS induced electrical arrest after less than 1 min at 5 °C and in less than 3 min at 36 °C. Futhermore, cells did not respond to electrical stimulation, and this cessation of electrical activity persisted until the solution in the bath was changed to Tyrode solution. The Em remained nearly normal for at least 30 min at both 36 °C and 5 °C. The Em in hearts treated with UW solution was consistent with theoretically calculated values. The characteristics of the cardioplegic solution determine the physicochemical changes that occur in the myocyte in several ways: 1) The composition of the cardioplegic solution affects the composition of the extracellular fluid, which is a major determinant of the Em. 2) Following depolarization of the myocyte, massive Na^+ influx occurs due to the magnitude of the change in the Em [10]. This Na^+ gradient across the sarcolemma induces Ca^{2+} influx secondarily. Pappano et al. [13] have reported that lowering the $[Na^+]o$ decreases Na^+ influx, which is a depolarizing current; hence hyperpolarization should take place. Prevention of myocardial Ca accumulation seen at the end of reperfusion in our study may be due to the lower Em against Na^+ and Ca^{2+} influx and also the difference in the $[Na^+]o$. 3) Low temperature decreases membrane fluidity and lowers the Em [12], and each enzyme has its own temperature specificity. However, hypothermia at 15 °C inactivates most membrane-bound enzymes. 4) It has been demonstrated that an acidic enviroment, which often occurs during ischemia, markedly diminishes inward Ca^{2+} and Na^+ currents [26], as well as Na^+–K^+–ATPase activity in cardiac muscle [18]. 5) Although most studies were performed using K^+-cardioplegia, i.e., depolarized conditions, Gibbons and Fozzard [7] have reported that the best recovery of developed left ventricular pressure was obtained using a solution of K^+ 15 mmol, Na^+ 30 mmol, and Ca^{2+} 1.2 mmol. 6) Local anesthetics have been shown to de-



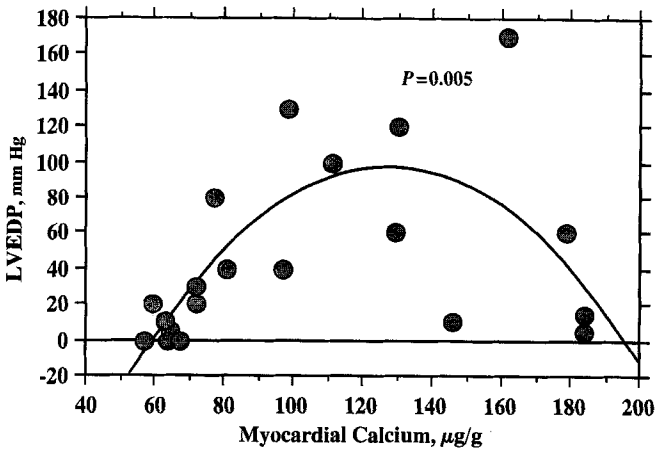


Fig. 3 Myocardial concentrations of Ca and ATP as a function of left ventricular end-diastolic pressure (*LVEDP*) following reperfusion after cardioplegia. *Left panel* Regression between LVEDP and myocardial ATP after 2 h reperfusion following 6- or 12 h preservation of canine hearts. $r = -0.65$, $n = 23$, $P = 0.004$. *ATP* Adenosine tri-phosphate. *Right panel* Regression between LVEDP and myocardial Ca after 2 h reperfusion following 6- or 12 h preservation of canine hearts. $r = -0.69$, $n = 23$, $P = 0.005$

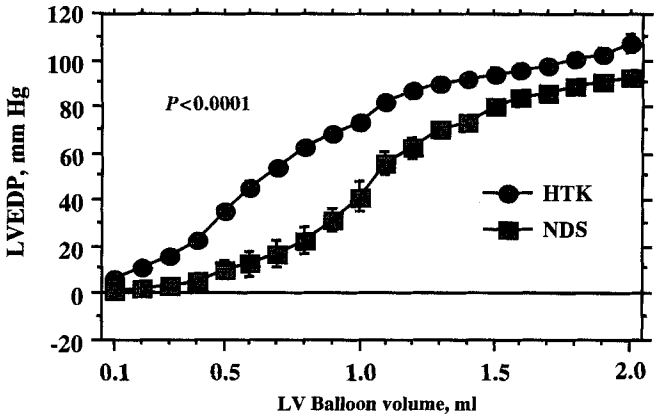


Fig. 4 Left ventricular end-diastolic pressure (*LVEDP*) – volume relationship after 30 min reperfusion following 30 min of cardioplegia at 37 °C comparing nondepolarizing solution (*NDS*) and Bretschneider's HTK solution. Data are presented as the mean; bars indicate SEM; $n = 10$ each group. *NDS* LVEDP was lower in NDS than HTK solution ($P < 0.0001$)

press the resting membrane conductance for K^+ and Na^+ and also to depress voltage-dependent slow channels [15]. Lidocaine acts as a Na-channel blocker [3]. These actions presumably represent a nonspecific effect of local anesthetics on membrane fluidity. Furthermore, local anesthetics also depress Na^+–K^+–ATPase activity [8]. 7) The osmolarity of the cardioplegic solution affects the immediate environment of the myocytes and may cause ion shifts that lead to changes in intracellular ion concentrations. In this study, we used a hypertonic solution with an osmolarity of 450 mOsm/l. Hypertonicity hyperpolarizes the membrane by 5–10 mV, depending upon the degree of hypertonicity [9, 1]. Finally, 8) the Na^+ concentration in the cardioplegic solution is believed to be a secondary factor to K^+ in determining the Em [17]. Our previous study [25] has shown that a cold solution with Na^+ 70 mM provides better myocardial protection than a solution with Na^+ 30 mM.

Sperelakis et al. [20] have reported in an *in vitro* study using cultured ventricular myocytes that a solution containing K^+ 5 mmol induces greater hyperpolarization than a solution with K^+ 0 mmol. We have found that the coronary bed contains blood which contains K^+, even though we intermittently flushed the heart with K^+-free solution [19], as K^+ moves from the intracellular to the extracellular space. Our observations suggest that even when we use a K^+-free solution, the extracellular K^+ concentration does not fall to zero and averaged about 5 mmol. This is one reason that we made our NDS K^+-free. Our finding suggests that: 1) NDS (K^+-free, low Na^+, lidocaine and hypertonic) induces electrical arrest at a normal Em and maintains nondepolarization for 30 min. Purkinje fibers and the myocytes *in situ* respond differently to an extracellular K^+ concentration of 0 mM than do single cells and tissue preservations. Purkinje cells depolarize at –20 mV after several minutes [5], and the level of depolarization in *in situ* myocytes varies as a function of the time of ischemia, and 2) it is impossible, both theoretically and practically, to maintain nondepolarization (polarization) of the myocardium for longer periods of ischemia using present techniques for cardioplegia, even though it has been reported that tetrodotoxin combined with cardioplegic solution can maintain normal Em, which results in better functional recovery in an *in vitro* study [22, 16].

Left ventricular diastolic function after ischemia-reperfusion

It has been reported that there are two components of ventricular diastolic dysfunction after reperfusion following ischemia: increased chamber stiffness, and decreased left ventricular relaxation [21]. These changes are due to hypoxia, depressed glycolysis, and an accumulation or disturbance in the compartmentalization of intracellular Ca^{2+} [2]. Our previous study [14] showed that the tissue Ca concentration correlates with both the elevation in the LVEDP and the decrease in left ventricular relaxation (LV –dp/dt). It also showed that the myocardial ATP concentration correlated directly with the max LV –dp/dt, but showed little correlation with the LVEDP, although the mycoardial Ca^{2+} concentration correlated with myocardial ATP depletion. From these findings and others [21, 27], we concluded that Ca overload leads directly to an increase in left ventricular chamber stiffness and disturbs the metabolic pathways of the high energy compounds. In turn, the decrease in the myocardial concentrations of high energy compounds decreases left ventricular relaxation. The present study validates these conclusions. In addition, our data show: 1) Nondepolarizing solution suppresses Ca^{2+} accumulation after reperfusion following prolonged preservation, 2) The myocardial Ca concentration is related to myocardial cAMP and cGMP concentrations, and 3) left ventricular diastolic function is better preserved in hearts preserved with nondepolarizing solution than in hearts treated with a depolarizing solution, such as UW or HTK. We are particularly interested in the correlation between myocardial concentrations of Ca to cAMP and cGMP. Lidocaine hydrochloride, a component of our NDS, is a sodium channel blocker and enhances polarization [15, 3, 8]. Lidocaine also activates protein kinase A (PKA), which leads to an increase in cAMP [28]. Whether the heart is depolarized or polarized affects not only the stiffness and relaxation of the ventricle during arrest, but also the basal metabolism and ion flux through membrane-bound channels. We have observed that the heart is very flaccid during cardiac arrest by nondepolarized solution. However, a parameter has yet to be developed which qualifies the flaccidity of the heart during arrest *in vivo*. However, the LVEDP after reperfusion was significantly lower in hearts treated with NDS than in hearts treated with HTK solution. We are unable to show a difference between these groups in the myocardial ATP concentration at the end of cardiac arrest, but the myocardial Ca concentration clearly was different in hearts preserved with UW solution and NDS. Therefore, the absolute value of the Em may affect the function of the sarcolemma.

In summary, our NDS maintains the Em closer to the physiologic value than does UW solution or Bretschneider's HTK solution during 30 min of cardioplegia, however, it raised the Em during hypothermic preservation. Although our data is based on experiments *in vitro*, the use of NDS better preserves left ventricular diastolic function than UW especially with regards to stiffness after reperfusion following a 6- or 12 h hypothermic preservation. This beneficial effect of NDS relative to HTK solution was also demonstrated in normothermia *in vitro*.

References

1. Akiyama T, Fozzard HA (1975) Influence of potassium ions and osmolarity on the resting membrane potential of rabbit ventricular papillary muscle with estimation of the activityand the activitycoefficient of internal potassium. Circ Res 37: 621
2. Alto LE, Dhalla NS (1979) Myocardial cation contents during induction of calcium paradox. Am J Physiol 237: H713-H719
3. Bean BP, Cohen CJ, Tsien RW (1983) Lidocaine block of cardiac sodium channels. J Gen Physiol 81: 613–642
4. Bessho M, Ohsuzu F, Yanagida S, Sakata N, Aosaki N, Tajima T, Nakamura H (1991) Differential extractability of creatine phosphate and ATP from cardiac muscle with ethanol and percholoric acid solution. Anal Biochem 92: 117–124
5. Callewaert G, Vereecke J, Verdonck F, Carmelliet E (1987) Isolated cardiac Purkinje cells. In: Noble D, Powell T (eds) Electrophysiology of single cardiac cells. Academic Press, London, pp 187–222
6. Flaherty JT, Schaff HV, Goldman RA, Gott VL (1979) Metabolic and functional effect of progressive degree of hypothermia during global ischemia. Am J Physiol 236: 839–845
7. Gibbons WR, Fozzard HA (1971) High potassium and low sodium contractures in sheep cardiac muscles. J Gen Physiol 58: 483
8. Henn BA, Sperelakis N (1968) Stimulative and protective action of Sr^{2+} on Na^+, K^+-ATPase from cultured heart cells. Biochim Biophys Acta 163: 415
9. Hermsmeyer K, Rulon R, Speelakis N (1972) Loss of the plateau of the cardiac action potential in hypertonic solutions. J Gen Physiol 59: 779
10. Krohn E, Stinner B, Fleckenstein M, Gebhard MM, Bretschneider HJ (1989) The cardioplegic solution HTK: effects on membrane potential, intracellular K^+ and Na^+ activities in sheep cardiac Purkinje fibers. Pfluegers Arch 415: 269–275
11. Martin DR, Scott DF, Downes GL, Belzer FO (1972) Primary cause of unsuccessful liver and heart preservation. Cold sensitivity of the ATPase system. Ann Surg 175: 111–117
12. McMurchie EJ, Raison JK, Cairncross KD (1973) Temperature-induced phase changes in membranes of heart: A contrast between the thermal response of poikilotherms and homeotherms. Comp Biochem Physiol 44: 1017–1026

13. Pappano AJ, Sperelakis N (1969) Spontaneous contraction of cultured heart cells in high K^+ media. Exp Cell Res 54: 58
14. Shirai T, Sunamori M, Suzuki A (1993) Left ventricular diastolic function of the reperfused postischemic donor heart. Surg Today (Jpn J Surg) 23: 902–907
15. Slou JC, Zerahn K (1959) Investigations on the effect of some local anesthetics and other amines on the active transport of sodium through the isolated short-circuit frog skin. Biochim Biophys Acta 35: 324
16. Snabaitis AK, Shattock MJ, Chambers DJ (1997) Comparison of polarized and depolarized arrest in the isolated rat heart for long-term preservation. Circulation 96: 3184–3156
17. Sperelakis N (1979) Origin of the cardiac resting potential. In: Berne R, Sperelakis N, Geiger SR (eds) Handbook of physiology, Section 2: The cardiovascular system, Vol I. American Physiological Society, Bethesda, p 208
18. Sperelakis N, Lee NC (1971) Characterization of Na^+, K^+-ATPase isolated from embryonic chick hearts and cultured chick cells. Biochim Biophys Acta 233: 562
19. Sperelakis N, McLean MJ (1978) Electrical properties of cultured heart cells. In: Kobayashi T, Ito Y, Rona G (eds) Recent Adv Cardiac Strucut Metab 12. University Park Press, Baltimore, p 645
20. Sperelakis N, Mayer G, MacDonald R (1970) Velocity of propagation in vertebrate cardiac muscles as functions of tonicity and $[K^+]o$. Am J Physiol 219: 952
21. Steenbergen C, Murphy E, Watts JA, London RE (1990) Correlation between cytosolic free calcium, contracture, ATP, and irreversible ischemic injury in perfused rat heart. Circ Res 66: 135–146
22. Sterbergh WC, Brunsting LA, Abd-Elfattah AS, Wechsler AS (1989) Basal metabolic energy requirements of polarized and depolarized arrest in rat heart. Am J Physiol 256: H846–H851
23. Sunamori M, Sultan I, Shirai T, Suzuki A (1992) The significant role of membrane stabilization in hypothermic cardioplegic cardiac preservation in a canine experimental model. Transpl Int 5 [Suppl 1]: 411–416
24. Sunamori M, Miyamoto H, Yoshida T, Suzuki A (1994) Successful cardiac preservation for 12 hours using nondepolarizing cold cardioplegia. A canine model. Transpl Int 7 [Suppl 1]: 458–488
25. Sunamori M, Shirai T, Amano J, Miyamoto H, Suzuki A (1994) The effect of sodium concentration on myocardial viability in donor heart preservation using a nondepolarizing solution. Transpl Int 7: 109–114
26. Vogel S, Sperelakis N (1977) Blockade of myocardial slow inward current at low pH. Am J Physiol 233: C99
27. Whitman GJR, Kieval RS, Brown J, Banerjee A, Grosso MA, Harken AH (1989) Optimal hypothermic preservation of arrested myocardium in isolated perfused rabbit hearts: A 31P NMR study. Surgery 105: 100–108
28. Wong JA, Man RYK, Choy PC (1994) The effect of lidocaine on denovo phospholipid biosynthesis in the isolated hamster heart. Lipids 29: 391–396