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

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 preparations. Postreperfusion myocardial cyclic adenosine monophosphate (cAMP) and adenosine triphosphate (ATP) concentrations in the NDS groups were closer to normal than in the UW groups after the 6- and 12 h preparations. The postreperfusion myocardial Ca concentration correlated with the cAMP (r = −0.68, n = 25, P = 0.003) 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 = 0.005). 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 normothermic 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 myocardial Ca concentration is inversely correlated with the cAMP and cGMP concentrations.

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

Abbreviations AMPP Action membrane potential · ATP Adenosin 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

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**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 preservation 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 ITHK solution or nondepolarizing solution were compared.

**Materials and methods**

Study of membrane resting potential in the Purkinje 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, CaCl2 1 mmol, MgL-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 osmolality 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, CaCl2 2 mmol, MgCl2 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 × 10¹⁰Ω. 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