CARDIOPULMONARY BYPASS, MYOCARDIAL MANAGEMENT, AND SUPPORT TECHNIQUES

PROTECTIVE EFFECTS OF DIMETHYL AMILORIDE AGAINST POSTISCHEMIC MYOCARDIAL DYSFUNCTION IN RABBIT HEARTS: PHOSPHORUS 31-NUCLEAR MAGNETIC RESONANCE MEASUREMENTS OF INTRACELLULAR pH AND CELLULAR ENERGY

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The effects of 5-(*N,N*-dimethyl)amiloride, a potent and specific Na⁺-H⁺ exchange inhibitor, were investigated in isolated perfused rabbit hearts subjected to ischemia and reperfusion. Phosphorus 31-nuclear magnetic resonance spectroscopy was used to monitor intracellular pH, creatine phosphate, β -adenosine triphosphate, and inorganic phosphate. After cardioplegic arrest with St. Thomas' Hospital solution, normothermic (37° C) global ischemia was induced for 45 minutes, and the hearts were reperfused for 50 minutes. Dimethyl amiloride at 10 μ mol/L, which has minimal inotropic and chronotropic effects on the nonischemic heart, was added to the cardioplegic solution. Treatment with dimethyl amiloride reduced the elevation of left ventricular end-diastolic pressure during and after the ischemia and improved the postischemic recovery of developed pressure from 76% $\pm$ 3.2% at 30 minutes of reperfusion in control hearts (*n* = 6) up to 99% $\pm$ 1.9% in hearts treated with dimethyl amiloride (*n* = 8). Dimethyl amiloride did not affect the decline in intracellular pH during ischemia for up to 30 minutes but enhanced the intracellular acidosis thereafter. The intracellular pH at the end of ischemia was 6.21 $\pm$ 0.05 in control hearts compared with 5.24 $\pm$ 0.17 in hearts treated with dimethyl amiloride (*p* < 0.05). During reperfusion, intracellular pH of hearts treated with dimethyl amiloride was less than control for 5 minutes, but subsequent recovery of intracellular pH was similar to control. Treatment with dimethyl amiloride did not affect creatine phosphate breakdown, inorganic phosphate accumulation, and β -adenosine triphosphate depletion during 45 minutes of ischemia. The creatine phosphate resynthesis and inorganic phosphate reduction during reperfusion were also unaffected. These findings suggest that Na⁺-H⁺ exchange plays an important role not only during reperfusion but also during ischemia for the development of postischemic cardiac dysfunction most likely by inducing primary Na⁺ and secondary Ca²⁺ overload. Specific Na⁺-H⁺ exchange inhibitors like dimethyl amiloride would have a potential therapeutic profile in cardiac surgery, especially if added before ischemia. (*J Thorac Cardiovasc Surg* 1996;112:765-75)

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Pharmacologic evidence for the involvement of sarcolemmal Na⁺-H⁺ exchange in myocardial ischemia-reperfusion injury has emerged from many experimental studies showing various salutary effects of Na⁺-H⁺ exchange inhibitors, including amiloride and other organic compounds.¹⁻³ In animal experiments in which rats, guinea pigs, rabbits, and dogs were used, many investigators have dem-

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onstrated that treatment of hearts with amiloride or other compounds having more specific inhibitory action against $\text{Na}^+\text{-H}^+$ exchange improves postischemic contractile recovery,^{4,7} reduces reperfusion arrhythmias,⁸ diminishes creatine kinase efflux during reperfusion,⁶ preserves microscopic cellular integrity,⁹ or reduces Na^+ and Ca^{2+} accumulation.^{4,10} Regarding the precise nature of their beneficial effects, however, several issues remain to be resolved.

Controversy exists as to whether cardiac protection by $\text{Na}^+\text{-H}^+$ exchange inhibitors is limited to reperfusion or would also occur under ischemic conditions.^{2,3} $\text{Na}^+\text{-H}^+$ exchange inhibitors were shown to have a certain protective effect if the compound was given only during reperfusion.⁶⁻⁹ Nevertheless, protection is also present during ischemia^{9,11,12} and is even more pronounced if the inhibitor is given during ischemia and reperfusion.⁹ The protective effects of $\text{Na}^+\text{-H}^+$ exchange inhibitors were associated with the preservation of high-energy phosphates in some studies,^{9,13} but not in others.^{4,10}

The cardioprotective effects of $\text{Na}^+\text{-H}^+$ exchange inhibitors have the potential to be clinically useful. In view of the preclinical data, the most promising indications for the inhibitors include the typical ischemia-reperfusion situation, in which the start of ischemia and reperfusion is known and sufficient concentrations of the compound can be applied to the heart. This is the case in cardiac surgery.³ During cardiac operations, the contraction of the heart has to be stopped by a cardioplegic solution. The heart is disconnected from systemic blood flow and is reperfused at the end of the procedure. Various protective drugs are, therefore, usually applied to the cardioplegic solution so that their concentration in the heart will remain high enough without affecting other organs. To our knowledge, however, little information is available as to the cardioprotective effects of $\text{Na}^+\text{-H}^+$ exchange inhibitors against ischemia-reperfusion injury when applied to the cardioplegic solution.

The current study was designed to gain further insight into the mechanism of cardioprotective action of $\text{Na}^+\text{-H}^+$ exchange inhibitors and their potential therapeutic profile in cardiac surgery. We investigated the effects of 5-(*N,N*-dimethyl) amiloride (DMA), a more potent and more specific inhibitor of $\text{Na}^+\text{-H}^+$ exchange than amiloride,^{8,14} on the sequential changes of left ventricular contractility, intracellular pH (pH_i), and high-energy phos-

phates in isolated perfused rabbit hearts subjected to normothermic global ischemia during cardioplegic arrest and subsequent reperfusion. DMA was applied to the cardioplegic solution, and pH_i and energy metabolism were monitored by use of phosphorus 31-nuclear magnetic resonance (NMR) spectroscopy.

Materials and methods

Preparation. Hearts were obtained from young white rabbits, 4 to 7 weeks old, weighing 500 to 800 gm. After intravenous administration of heparin (1000 IU) and thiamylal sodium (20 mg/kg), hearts were quickly excised and placed in cold (4° C) perfusion solution. The aorta was cannulated for nonrecirculating Langendorff perfusion at a constant hydrostatic pressure (75 cm H_2O). The control perfusate was a modified phosphate-free Krebs-Henseleit bicarbonate buffer solution of the following composition (in millimoles per liter): NaCl, 118; KCl, 5.9; NaHCO_3 , 25; MgSO_4 , 1.2; CaCl_2 , 2.5; and glucose, 5.5. The solution was equilibrated with 95% oxygen and 5% carbon dioxide to achieve a pH of 7.4 at 37° C. A latex balloon (size 4.3 mm in maximal diameter and 6.3 mm in length, Hirokawa, Niigata, Japan) was introduced into the left ventricle via the left atrium and connected to a strain-gauge transducer (MIP-5100, Baxter, Tokyo, Japan) for measurement of isovolumic left ventricular pressure. The balloon was filled with distilled water containing a 0.5 mol/L concentration of methylene diphosphonic acid as external standard of the NMR spectral reference. The left ventricular end-diastolic pressure was adjusted to 5 mm Hg during the equilibration period in each heart, and the volume was unchanged during the experiments. The left ventricular developed pressure was calculated by subtracting left ventricular end-diastolic pressure from left ventricular systolic pressure. The maximum rate of contraction and relaxation ($\pm \text{dp}/\text{dt}$) was obtained by electronic differentiation. The pulmonary artery was incised to ensure adequate right ventricular venting. Myocardial temperature was maintained at $37^\circ \pm 0.5^\circ \text{C}$ by a water-jacketed perfusion line and a continuous stream of air around the sample tube of NMR.

All animals have received humane care in compliance with 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 Institute of Laboratory Animal Resources and published by the National Institutes of Health (NIH publication No. 86-23, revised 1985).

NMR spectroscopy. In all the ischemia-reperfusion experiments, ^{31}P -NMR spectra were monitored along with simultaneous recording of ventricular pressure. The heart connected to the Langendorff perfusion line was placed in a standard 20 mm NMR tube with the apex approximately 2.5 cm from the bottom of the tube, and the tube was inserted into the NMR coil. The effluent was removed from a level above the heart by a peristaltic pump, leaving the heart submerged in a fixed volume of the perfusate.

^{31}P -NMR spectra were obtained at 161.8 MHz on a GSX 400 spectrometer (Jeol Datum Co. Ltd., Tokyo,

Fig. 1. ^{31}P -NMR spectra obtained from an untreated (control) rabbit heart before ischemia, during ischemia, and during reperfusion. Numbered peaks are as follows: 1, methylene diphosphate; 2, intracellular Pi; 3, PCr; 4, 5, and 6, γ -, α -, and β -phosphate group of ATP, respectively.

Japan) equipped with a 9.4-tesla vertical-bore magnet. For each spectrum, 90 free-induction decays (4 minutes) were accumulated after 45-degree flip-angle pulses (18 μsec) by use of 4096 data points and 15.015 kHz spectral width at a repetition time of 2 seconds. Accumulated free-induction decays were exponentially filtered, resulting in 30 Hz line broadening. Quantification of inorganic phosphate (Pi), phosphocreatine (PCr), and β -adenosine triphosphate (ATP) in the myocardium was achieved by integration of areas under individual peaks of interest in each spectrum using a specially designed gaussian line fitting software (Datum Station ALICE, Jeol Datum) installed in a computer (NEC 9801, NEC Co., Ltd., Tokyo, Japan).¹⁵ Values of PCr and ATP were expressed as a percentage relative to the individual spectrum obtained during the preischemic control period. Pi was expressed as a percentage of the sum of phosphate from PCr, ATP, and Pi during preischemic control perfusion¹⁶ by the equation: $(\text{Pi}/[\text{PCr} + 3\text{ATP} + \text{Pi}]_{\text{preischemic}}) \times 100\%$.

pH_i was measured by the chemical shift of the Pi peak relative to the PCr peak. The chemical shift values in parts

per million were converted to pH units as previously described^{17, 18} (Fig. 1).

Protocols

Effects of DMA on mechanical performance of nonischemic hearts. Hearts were allowed to beat spontaneously with a rate ranging from 147 to 174 beats/min. After a stabilization period of 20 to 30 minutes, the perfusate was changed from the control to a solution including DMA at a concentration of 10 $\mu\text{mol/L}$. Effects of DMA on heart rate, left ventricular systolic, end-diastolic, and developed pressure, $+\text{dp}/\text{dt}$, and $-\text{dp}/\text{dt}$ were monitored during 45 minutes of exposure to DMA and 30 minutes after the compound was washed out.

Influence of DMA on the hearts subjected to ischemia and reperfusion. After a stabilization period in the test tube of the NMR spectrometer for 20 to 30 minutes, preischemic values for left ventricular systolic, end-diastolic, and developed pressures, $\pm\text{dp}/\text{dt}$, and heart rate were recorded. The hearts were then perfused with St. Thomas' Hospital cardioplegic solution at 37° C at a constant pressure (75 cm H₂O). The composition of the solution was as follows (in millimoles per liter): NaCl,

Fig. 2. Changes in the left ventricular end-diastolic pressure (*LVEDP*) during 45 minutes of ischemia and subsequent 50 minutes of reperfusion. Left ventricular end-diastolic pressure is plotted as a function of time. Zero time is the beginning of cardioplegia and the beginning of reperfusion. Values are presented in means $\pm$ standard error of six untreated hearts (control, open circles) and eight hearts treated with DMA at 10 μ mol/L (closed circles). *Significantly different from control at $p < 0.05$.

110; KCl, 16; MgCl₂, 16; CaCl₂, 1.2, and NaHCO₃, 10.0 (pH 7.8). DMA was added to the St. Thomas' Hospital cardioplegic solution at 10 μ mol/L. The cardioplegia line was then clamped and global ischemia was produced for 45 minutes at normothermia (37° C). The hearts were bathed in the cardioplegic solution to avoid oxygen diffusion from the surrounding air. After ischemia, the hearts were reperfused with the control perfusate at 37° C for 50 minutes. During the ischemia-reperfusion insult, hemodynamic measurements were repeated to document functional deterioration and recovery. The hearts were allowed to beat spontaneously throughout the experiments.

Effects of DMA on hearts subjected to ischemia and reperfusion. Fourteen hearts subjected to normothermic cardioplegic arrest plus ischemia and reperfusion were divided into two groups. In six control hearts, normal St. Thomas' Hospital cardioplegic solution was used for cardioplegia before ischemia. In the remaining eight hearts, DMA was added to the cardioplegic solution in a concentration of 10 μ mol/L.

Data analysis. All the data were presented as means $\pm$ standard error unless otherwise specified. Hemodynamic data in experiments with nonischemic hearts were expressed as a percentage of pre-drug control values. In ischemia-reperfusion experiments, preischemic function was expressed as an absolute value for each parameter. Postischemic recovery of cardiac function was expressed as an absolute value and as a percentage of its preischemic level. We analyzed NMR data from 15 minutes before ischemia to up to 30 minutes of reperfusion because of a limitation of sam-

pling and processing capacity of our computer system, and because hemodynamic recovery of both control and DMA-treated hearts reached almost plateau levels at 30 minutes of reperfusion (Figs. 2 and 3). The unpaired *t* test was used for comparison of means between the groups. Statistical significance was defined as $p < 0.05$.

Results

Effects of DMA on left ventricular contractility in nonischemic hearts. Treatment of the hearts with DMA at 10 μ mol/L resulted in a gradual decrease in heart rate. Heart rate at the end of 45 minutes' exposure to DMA reached 67% $\pm$ 10.0% of the control level ($n = 5$, $p < 0.05$). Average values of left ventricular systolic and developed pressures, +dp/dt, and -dp/dt were virtually unaffected by the DMA treatment. On washing out of DMA for 30 minutes, heart rate recovered to 86% $\pm$ 6.7% of control ($p < 0.05$). No appreciable changes in left ventricular end-diastolic pressure occurred throughout the exposure and washing out of DMA.

Hemodynamic function. Averages of heart rate, left ventricular systolic and developed pressures, +dp/dt, and -dp/dt under preischemic condition are summarized in Table I. No significant differences were observed in these baseline parameters between the control and DMA-treated groups.

Fig. 3. Changes in the left ventricular systolic pressure (LVSP), developed pressure (LVDP), and maximum rate of contraction and relaxation ($\pm dp/dt$) during 45 minutes of ischemia and a subsequent 50 minutes of reperfusion. The hemodynamic parameters are plotted as a function of time. Zero time is the beginning of cardioplegia and the beginning of reperfusion. Values are means $\pm$ standard error of six untreated hearts (control, open circles) and of eight hearts treated with DMA at 10 $\mu\text{mol/L}$ (closed circles). *Significantly different from control at $p < 0.05$.

Table 1. Baseline hemodynamic parameters during the preischemic condition

	Control group (n = 6)	DMA group (n = 8)
HR (beats/min)	168 ± 3.3	157 ± 3.1
LVDP (mm Hg)	72 ± 2.6	68 ± 6.0
LVSP (mm Hg)	77 ± 2.6	63 ± 6.0
+dp/dt (mm Hg/sec)	1800 ± 67	1790 ± 64
-dp/dt (mm Hg/sec)	1330 ± 79	1480 ± 53

HR, Heart rate; DMA, dimethyl amiloride; LVDP, left ventricular developed pressure; LVSP, left ventricular systolic pressure. There was no significant difference between the groups. Values are means ± standard error of the mean.

Sequential changes in left ventricular end-diastolic pressure during and after ischemia are shown in Fig. 2. In the control group ($n = 6$), left ventricular end-diastolic pressure increased progressively during the 45-minute period of ischemia. When the hearts were reperfused, end-diastolic pressure further increased temporarily, giving rise to a peak at 1 minute of reperfusion. Pressure then decayed rather quickly toward the preischemic level within 20 minutes. Left ventricular end-diastolic pressure in the DMA-treated hearts ($n = 8$) also increased during and immediately after ischemia with a similar time course, but the extent of increase was significantly less than control.

Fig. 3 summarizes the changes in left ventricular systolic and developed pressures and $\pm dp/dt$ during and after ischemia. In both control and DMA-treated hearts, left ventricular systolic and developed pressures decreased rapidly after initiation of cardioplegia, and the mechanical contraction became undetectable within 1 minute. On reperfusion, left ventricular systolic and developed pressures showed dual phases of recovery; initial rapid recovery within 5 minutes was followed by much slower recovery thereafter. In the control group ($n = 6$), the initial rapid recovery of left ventricular systolic and developed pressures after ischemia peaked at 3 minutes of reperfusion. Then left ventricular systolic and developed pressures decayed a little before the subsequent slower recovery. At 30 minutes of reperfusion, they reached a plateau at $82.4\% \pm 5.4\%$ and $75.6\% \pm 3.2\%$, respectively. In the DMA-treated group ($n = 8$), recovery of left ventricular systolic and developed pressures during reperfusion was slower during the initial 5 minutes, but it was significantly greater during the subsequent period, reaching $99.5\% \pm 1.7\%$ and $99.9\% \pm 1.9\%$ of their respective preischemic levels at 30 minutes of reper-

fusion ($p < 0.05$ vs control). During reperfusion later than 30 minutes, left ventricular systolic and developed pressures further increased, and at 50 minutes they reached 5% to 11% above their preischemic levels.

Comparative differences between control and DMA-treated hearts were observed in the recovery of $\pm dp/dt$. At 5 minutes of reperfusion, recovery of $+dp/dt$ and $-dp/dt$ in the DMA-treated group toward the baseline was significantly less than that of the control group. At 30 minutes' reperfusion, however, the recovery of $+dp/dt$ and $-dp/dt$ in the DMA-treated group was significantly higher than in the control group.

In both the control and DMA-treated hearts, heart rate recovered nearly completely by 10 minutes of reperfusion, and there was no significant difference between the two groups.

pH_i. Changes in pH_i during and after ischemia are shown in Fig. 4. The initial (preischemic) level of pH_i was almost identical in control (7.25 ± 0.04 , $n = 6$) and DMA-treated hearts (7.26 ± 0.05 , $n = 8$). An infusion of St. Thomas' Hospital solution resulted in a transient increase in pH_i in both control (up to 7.56 ± 0.05) and DMA-treated hearts (up to 7.67 ± 0.08), probably because of the higher pH (7.8) of the cardioplegic solution and PCr breakdown (see Discussion).

During the subsequent period of ischemia, pH_i decreased progressively; its reduction was similar in both groups for 30 minutes of ischemia, but significantly greater in the DMA-treated group than in the control group thereafter. The pH_i at the end of 45 minutes of ischemia was 6.21 ± 0.05 in control hearts and 5.24 ± 0.17 in the DMA-treated hearts. During reperfusion, pH_i in both control and DMA-treated hearts increased rapidly and returned to the preischemic level within 15 minutes. For the first 5 minutes of reperfusion, pH_i of the DMA-treated hearts was lower than control, but there was no significant difference in the levels during the subsequent period of reperfusion.

High-energy phosphates. Changes in PCr, Pi, and ATP levels during and after 45 minutes of ischemia are shown in Fig. 5. PCr and ATP values are presented as percentages relative to the preischemic baseline, whereas Pi values are expressed as percentages of the sum of phosphates during the preischemic period.¹⁶ During ischemia, the rapid degradation of PCr was balanced by an increase in intracellular Pi. A moderate decrease in ATP was observed as well.

Fig. 4. Changes in pH_i during 45 minutes of ischemia and a subsequent 30 minutes of reperfusion. Ordinate indicates pH_i calculated from the chemical shift of Pi peak from PCr peak in ^{31}P -NMR spectra. Abscissa is time (in minutes) after the initiation of cardioplegia with St. Thomas' Hospital cardioplegic solution and after the initiation of reperfusion. Values are means $\pm$ standard error of the mean of six untreated hearts (control, open circles) and eight hearts treated with DMA at $10 \mu\text{mol/L}$ (closed circles). *Significantly different from control at $p < 0.05$.

On reperfusion, PCr was rapidly resynthesized to levels above the preischemic baseline in both control and DMA-treated groups. In association with this change in PCr, Pi decreased rapidly to the preischemic baseline. In contrast, there was no appreciable change in ATP levels during the 30-minute reperfusion period, and there were no significant differences between control and DMA-treated groups in these sequential changes of high-energy phosphates during and after ischemia.

Discussion

DMA. In the present study we used DMA as a pharmacologic tool to inhibit $\text{Na}^+\text{-H}^+$ exchange in cardiac cell membrane, because of its high potency and high specificity. DMA is 10 to 24 times more potent than amiloride in inhibition of $\text{Na}^+\text{-H}^+$ exchange, but it is less effective than amiloride in other intrinsic actions unrelated to the antiport.^{8,14} The dose of DMA used in our experiments ($10 \mu\text{mol/L}$) is close to the 50% inhibitory concentration for DMA to block $\text{Na}^+\text{-H}^+$ exchange in sarcolemmal vesicles, but much less than the 50% inhibitory concentration to block $\text{Na}^+\text{-Ca}^{2+}$ exchange (550

$\mu\text{mol/L}$), Na/K ATPase ($3000 \mu\text{mol/L}$), voltage-gated Na^+ channel ($>50 \mu\text{mol/L}$), and voltage-gated Ca^{2+} channel ($>50 \mu\text{mol/L}$).^{8,14} DMA also has an advantage over other amiloride analogs in terms of its specificity.¹⁵

We did not pace the hearts at a constant rate for the sake of simplicity of equipment around the NMR tube. Instead, the hearts were allowed to beat spontaneously throughout each ischemia-reperfusion experiment. This may not invalidate the effects of DMA, because spontaneous beating was terminated by cardioplegia at the onset of ischemia, and there was no significant difference in heart rate between control and DMA-treated groups during the entire period of reperfusion.

The protective actions of DMA against ischemia-reperfusion damage to contractility in the present experiments are qualitatively similar to those reported by Meng and associates.^{6,20,21} In experiments using coronary-perfused rat ventricular muscles, they showed that treatment of the preparations with DMA (1 to $20 \mu\text{mol/L}$) before 60 minutes of ischemia and at the time of reperfusion reduced the elevation of the resting tension and improved the

Fig. 5. Changes in high-energy phosphates during 45 minutes of ischemia and a subsequent 30 minutes of reperfusion. *Ordinates* indicate PCr in A, Pi in B, and ATP in C. *Abscissas* indicate time (in minutes) after the initiation of cardioplegia with St. Thomas' Hospital cardioplegic solution and after the initiation of reperfusion. PCr and ATP were expressed as a percentage relative to the preischemic baseline. Pi was expressed as a percentage of the sum of phosphate from PCr, ATP, and Pi during the preischemic baseline period. Values are means $\pm$ standard error of the mean of six untreated hearts (control, open circles) and eight hearts treated with DMA at 10 μ mol/L (closed circles).

recovery of contractility in a concentration-dependent manner. DMA (10 to 50 $\mu\text{mol/L}$) was also reported to reduce reperfusion arrhythmias⁸ and to reduce the intracellular Ca^{2+} accumulation during ischemia in rat hearts.¹⁰ To our knowledge, however, this is the first report to demonstrate that DMA, when added to cardioplegic solution, protects against ischemia-reperfusion injury in mammalian hearts at a concentration with minimal intrinsic actions.

pH_i. The pH_i at the beginning of ischemia (immediately after infusion of St. Thomas' Hospital cardioplegic solution) increased slightly both in control hearts (from 7.25 to 7.57) and in DMA-treated hearts (from 7.26 to 7.67). This transient alkalosis may be attributed in part to the higher pH level (7.8) of the cardioplegic solution. A rapid breakdown of PCr, which absorbs H^+ , in the initial period of ischemia (see Fig. 5) may also help to produce this transient alkalosis because it increases intracellular H^+ buffering capacity.¹ pH_i then decreased progressively during the subsequent period of ischemia. DMA treatment did not affect this pH_i reduction for the initial 30 minutes, but it enhanced the intracellular acidosis during the later period of ischemia. At the end of the 45-minute period of ischemia in the DMA-treated hearts, pH_i (5.24) was 1.0 unit lower than in control hearts; it recovered rapidly in both control and DMA-treated hearts and returned to the preischemic level by 10 to 15 minutes. In the DMA-treated group during reperfusion, pH_i was lower than control only at the initial sampling (4 minutes). These findings differ from those of previous reports on the effects of pharmacologic block of Na^+-H^+ exchange on pH_i during ischemia-reperfusion injury.

In ³¹P-NMR studies using hearts from rats and ferrets, preischemic treatment with amiloride or 5-(*N*-ethyl-*N*-isopropyl)amiloride (EIPA) was shown to delay pH_i recovery during early reperfusion without affecting the progressive pH_i decline during the preceding period of ischemia.^{5,13} Hendriks and associates⁹ also demonstrated in rabbit hearts that HOE 694 (a new specific Na^+-H^+ exchange inhibitor) did not affect the pH_i decline during ischemia, but eliminated the alkaline overshoot at the onset of reperfusion.

The discrepancy between these previous reports and our data may be related to complex regulatory mechanisms of intracellular H^+ concentration. During ischemia, H^+ is produced in the cytosol mainly from glycolytic ATP turnover, carbon dioxide retention, and later net ATP degradation.¹ In the initial

period of ischemia, protons are first buffered by intracellular protein histidine residues and by Pi, which is a product of PCr breakdown to help maintain ATP levels. Unbuffered intracellular H^+ can leave via sarcolemmal $\text{H}^+-\text{lactate}$ cotransporter, Na^+-H^+ exchange, and $\text{Na}^+-\text{HCO}_3^-$ coinflux.^{1,22} As ischemia progresses, however, further H^+ efflux is likely to be opposed by interstitial H^+ accumulation, leading to a decrease of transsarcolemmal H^+ gradient, and by intracellular Na^+ accumulation, caused by an inhibition of sarcolemmal Na/K ATPase.³ Competition of H^+ and Ca^{2+} in the intracellular binding sites²³ also affects the free H^+ concentration in the cytosol. Accumulation of intracellular Ca^{2+} during ischemia thus favors a further decrease in pH_i through displacement of H^+ from intracellular buffering sites by Ca^{2+} . An active Na^+-H^+ exchange process is not necessarily related to pH regulation, because coupling of this process with $\text{Cl}^--\text{HCO}_3^-$ exchange (Cl^- influx) results in zero acid extrusion, and net NaCl influx, reported to be a mechanism for volume regulation.^{24,25} A decrease of H^+ influx via Na^+-H^+ exchange was reported to reduce the H^+ production by anaerobic glycolysis.^{11,26} The ultimate effect of pharmacologic inhibition of Na^+-H^+ exchange on pH_i, therefore, depends on the relative importance of this antiport in the total capacity of H^+ buffering and extrusion and on the secondary modulation of intracellular H^+ generation.

The activity of Na^+-H^+ exchange is known to be inhibited by lowering the extracellular pH.^{27,28} The pH of the cardioplegic solution used in the present study (7.8) is higher than that of ordinary buffer perfusates (7.4) used by other investigators. This might have helped to maintain greater Na^+-H^+ exchange activity during ischemia in our experiments. Other conditions that increase the Na^+-H^+ exchange activity, such as sufficient transsarcolemmal Na^+ gradient or phosphorylation of the exchanger via protein kinase C, might have been different between the present experiments and the previous studies.² If the relative contribution of Na^+-H^+ exchange to the net H^+ buffering and extrusion capacity of cardiac muscle is small, an inhibition of the antiport will be easily masked by other multiple regulatory mechanism as described earlier and will result in no observable change in pH_i.

On reperfusion, a prompt washout of extracellular H^+ and metabolites is thought to lead to a rapid efflux of accumulated intracellular H^+ through a passive efflux of lactate and carbon dioxide, as well

as an active H^+ extrusion mechanism (Na^+-H^+ exchange and $Na^+-HCO_3^-$ coinflux).^{3,22} In the present experiments, treatment with DMA caused only a slight delay in pH_i recovery during reperfusion, suggesting a relatively small contribution of Na^+-H^+ exchange to the pH_i recovery during reperfusion. In a recent extensive study using ferret hearts, Vandenberg, Metcalfe, and Grace²² have concluded that the pH_i recovery during reperfusion is principally mediated by metabolite washout, that is, lactate and carbon dioxide extrusion, and that Na^+-H^+ exchange and HCO_3^- influx may contribute as much as 20% to 35% of net H^+ efflux.

During ischemia, the exchanger is partially inhibited by low extracellular pH ,^{27,28} but this inhibition can be overdriven by intracellular acidosis.²⁸ A rise in intracellular Na^+ during ischemia and its suppression by amiloride or EIPA have been documented by ^{23}Na -NMR.^{10,13} A rise in cytosolic Ca^{2+} during ischemia and its suppression by amiloride were also demonstrated in ^{19}F -NMR.¹⁰ The present data indicating an enhancement of pH_i reduction and an attenuation of left ventricular end-diastolic pressure elevation in DMA-treated hearts from the later period of ischemia to early reperfusion are in agreement with those of previous reports suggesting an important role of Na^+-H^+ exchange during ischemia. Na^+-H^+ exchange blockers may improve the functional recovery of cardiac muscle by limiting the Na^+ gain during ischemia and by inhibiting the additional rise of Na^+ on reperfusion when the partial block of external acidification on Na^+-H^+ exchange is suddenly relieved. Intracellular acidification during the early period of reperfusion would also favor the cardiac muscle by inhibition of Ca^{2+} entry via Na^+-Ca^{2+} exchange, coupling of Ca^{2+} to contractile elements, and other Ca^{2+} -induced reactions.³

High-energy phosphates. There is considerable controversy among previous reports whether the protective effects of Na^+-H^+ exchange blockers are associated with an improvement of energy metabolism. In experiments using rat hearts, some investigators showed that amiloride had no significant effects on the reduction of ATP and PCr during ischemia and reperfusion.^{4,10} In experiments by other investigators using rat or rabbit hearts,^{9,13} however, EIPA or HOE 694 was shown to reduce ATP depletion during ischemia and to improve recovery of PCr and ATP during reperfusion. In the present experiments, preischemic treatment with DMA did not affect PCr breakdown, Pi accumula-

tion, or ATP depletion during 45 minutes of ischemia. The PCr resynthesis and Pi reduction during reperfusion were unaffected as well.

The reason for the different observations is unclear. Depletion of high-energy phosphates during ischemia and reperfusion could be ameliorated by an inhibition of Na^+-H^+ exchange via a reduction of intracellular Na^+ and Ca^{2+} overload,^{2,3} because a great deal of ATP is used for extrusion of Na^+ and for storage of Ca^{2+} in the sarcoplasmic reticulum. Ca^{2+} overload also activates ATP use by many ATPases and inhibits mitochondrial oxidative phosphorylation to produce ATP.^{29,30} The increase of left ventricular end-diastolic pressure, which reflects Ca^{2+} overload, and the decrease in ATP observed in our control experiments of ischemia and reperfusion are appreciably less than those reported by Hendriks,⁹ Pike,¹³ and their associates. This is probably due to the cardioprotective action of St. Thomas' Hospital cardioplegic solution in our experiments. Beneficial effects of Na^+-H^+ inhibition on the energy metabolism might be detected only when the Na^+ and Ca^{2+} overload is above a certain critical level. This issue remains to be tested.

Conclusion

The present study has revealed that an application of DMA at an appropriate concentration to cardioplegic solution at the onset of ischemia causes a good improvement of ventricular function during the subsequent period of reperfusion. This beneficial effect is associated with an enhancement of pH_i reduction during late ischemia and early reperfusion but no appreciable modification of energy metabolism. It is suggested that Na^+-H^+ exchange plays an important role not only during reperfusion but also during ischemia for the development of postischemic cardiac dysfunction, most likely by inducing intracellular Na^+ and Ca^{2+} overload.

DMA seems to have an interesting therapeutic potential in cardiac surgery to ameliorate postarrest cardiac dysfunction or postischemic myocardial stunning. More extensive experimental studies, however, will have to be carried out before the exact clinical usefulness of this compound can be discussed. For instance, in cardiac operations in human beings, unlike the present *in vitro* experiments, the hearts are reperfused with blood. Many experimental and clinical studies have shown that Na^+-H^+ exchange plays important roles in activation of platelets and neutrophils, which are known to release various cardiotoxic factors on reperfusion such

as arachidonic acid, thromboxane A_2 , and leukotriene B_4 .² If these processes are inhibited by DMA, its beneficial effects against ischemia-reperfusion injury would be amplified. DMA might also modulate the volume regulation process^{24, 25} of blood cells and cardiac cells by inhibiting Na^+-H^+ exchange. These aspects of the drug action remain to be elucidated.

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