EFFECTS OF CARDIOPLEGIA ON MYOCARDIAL METABOLISM
DURING HYPOTHERMIC ISCHEMIA

— Components and mode of administration —

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Isolated rat heart preparations were used to determine the effect of cardioplegia on myocardial metabolism during profound hypothermic (15°C) ischemia. The hearts were grouped according to the components of cardioplegia and the mode of administration. The six groups were normokalemic (GI), calcium-containing hyperkalemic (GII), calcium-free hyperkalemic (GIII) and single dose (A), multidose (B). Following 120 min of ischemia, tissue ATP decreased from 25.4 ± 2.2 to 10.3 ± 2.7, 3.9 ± 2.4, 4.1 ± 1.2, 15.5 ± 3.2, 14.5 ± 2.4 and 20.0 ± 2.7 (I-A vs II-A p < 0.005, I-A vs III-A p < 0.005, I-B vs III-B p < 0.05, II-B vs III-B p < 0.005), and tissue lactate increased from 9.6 ± 1.5 to 163.4 ± 12.0, 174.1 ± 13.5, 166.8 ± 21.3, 99.1 ± 8.3, 102.6 ± 12.2 and 83.5 ± 9.3 (I-B vs III-B p < 0.02, II-B vs III-B p < 0.02) μmol/dry wt g, in GI-A, GII-A, GIII-A, GI-B, GII-B and GIII-B, respectively.

The results of this study suggest that (1) potassium cardioplegia in a single dose does not prevent degradation of high energy phosphate (HEP) in the hypothermic arrested heart, (2) though multidose cardioplegia is effective in preserving HEP during ischemia, the extent of its effects varies with the composition, and (3) the omission of calcium is beneficial in GIK cardioplegia in terms of preserving HEP at the end of ischemia.

Surgeons often induce cardiac arrest by aortic cross-clamping during open heart surgery. As prolonged ischemic cardiac arrest leads to myocardial damage, special techniques for protection of the myocardium including cardioplegia and topical hypothermia must be used. Numerous attempts have been made to search for the “ideal” cardioplegic solution and to compare different modes of cardioplegia administration. In the present study we measured the contents of high energy phosphates (HEP) and glycolytic intermediates in the ischemic myocardium in order to answer the following three questions.

(1) Does hyperkalemic cardioplegia affect the myocardial metabolism during profound hypothermic ischemia?
(2) Which is the better, single or multidose cardioplegia?
(3) Does the omission of calcium from cardioplegia lead to energy conservation during ischemia?

Key Words:
Myocardial ischemia
Myocardial metabolism
Cardioplegia
Calcium ion

(Received December 7, 1983; accepted October 23, 1984)
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This study was supported in part by Research Grant 57480282 from the Ministry of Education, Japan.

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TABLE I COMPOSITION OF SOLUTIONS INJECTED INTO THE AORTIC ROOT

<table>
<thead>
<tr>
<th></th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Krebs-Henseleit bicarbonate buffer solution (KHB)</td>
<td>Modified glucose-insulin-potassium solution (GIK)</td>
<td>Calcium-free GIK</td>
</tr>
<tr>
<td>NaCl</td>
<td>118 mM</td>
<td>saline</td>
<td>saline</td>
</tr>
<tr>
<td>KCl</td>
<td>4.7 mM</td>
<td>5% glucose</td>
<td>5% glucose</td>
</tr>
<tr>
<td>CaCl₂</td>
<td>2.0 mM</td>
<td>KCl</td>
<td>KCl</td>
</tr>
<tr>
<td>EDTA-Ca₂-2Na</td>
<td>0.5 mM</td>
<td>insulin</td>
<td>insulin</td>
</tr>
<tr>
<td>MgSO₄·7H₂O</td>
<td>1.2 mM</td>
<td>Calcium gluconate</td>
<td>Calcium gluconate</td>
</tr>
<tr>
<td>KH₂PO₄</td>
<td>1.2 mM</td>
<td>lidocaine</td>
<td>lidocaine</td>
</tr>
<tr>
<td>NaHCO₃</td>
<td>25 mM</td>
<td>NaHCO₃</td>
<td>NaHCO₃</td>
</tr>
<tr>
<td>glucose</td>
<td>11.0 mM</td>
<td></td>
<td></td>
</tr>
<tr>
<td>lactate</td>
<td>1.0 mM</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

MATERIALS AND METHODS

Sixty hearts from male Wistar rats weighing 250 to 350g were used. The rat was anesthetized with Nembutal (50 mg/rat i.p.), the femoral vein exposed, and heparin (200 i.u.) given intravenously. One minute after the administration of heparin, the heart was attached to a perfusion apparatus and retrograde perfusion down the aorta was initiated. Perfusion apparatus and perfusate used in this study were the same as reported previously. The heart was converted to a working mode by initiating left atrial perfusion at a pressure of 18 cmH₂O and ejected against a pressure of 60 mmHg for 10 min. Ischemic arrest was induced by clamping both aortic and left atrial canulasa, and myocardial temperature was lowered to 15°C by topical cooling.

After aortic cross-clamping, the hearts were divided into three groups according to the solution injected into the aortic root, the components of which are presented in Table I. Each group was then divided into two dosage subgroup A and B. Hearts in subgroup A received 3 ml of cold solution only at the onset of ischemia (single dose administration) and hearts in subgroup B received the same amount of solution initially and every 30 min during ischemia (multidose administration). Tissue samples for metabolic studies were obtained prior to the onset of ischemia (6 hearts) and after 10 min (6 hearts x 3 groups) and 120 min (6 hearts x 6 groups) of ischemia.

The whole hearts were rapidly frozen with Wollenberger tongs precooled in liquid nitrogen, and then stored at -70°C until analysed. The levels of following high energy phosphates and glycolytic intermediate were determined according to standard procedures using the NADH-coupled reaction; creatine phosphate (CP), adenosine triphosphate (ATP), adenosine diphosphate (ADP), adenosine monophosphate (AMP), total adenine nucleotide (TAN), glucose-6-phosphate (G6P), and lactate. All values are expressed as micromoles per gram dry weight, and all results are expressed as mean ± standard deviation. P-values were calculated from Student's t-test, using standard procedures for unpaired comparisons.

RESULTS

The hearts in Group I arrested 5 to 6 min following aortic cross-clamping, and hearts beat for 5 to 6 min after an injection of KHB solution. In contrast, the hearts in Group II and III ceased beating as soon as the cardioplegic solution was injected, and an additional injection of solution did not produce contraction. Tissue levels of metabolic intermediates under various conditions are presented in Fig. 1.

(i) Following 10 min of ischemia

A prompt reduction in tissue CP and about 300 percent increase of the control value in lactate were observed in all groups. However, tissue levels of ATP, TAN and ADP were not significantly changed. The value of AMP was significantly higher in Group I (p < 0.005) than in Group II, probably reflecting the conversion of ATP into AMP through the adenylate kinase reaction, since a slight change in ATP concentration can be amplified manifold with increase in AMP concentrations.

Fig 1. Tissue levels of metabolic intermediates during ischemia.

- Pre-ischemic value: ▲▼ Group I-A (single dose, KHB); ▲▼ Group I-B (multi
dose, KHB); ●● Group II-A (single dose, GIK); ●● Group II-B (multi dose,
GIK); ○○ Group III-A (single dose, Ca-free GIK); ○○ Group III-B (multi dose,
Ca-free GIK).

Legend: C = pre-ischemic value, 10 = following 10 min of ischemia, 120 = following 120 min of ischemia.

(ii) Following 120 minutes of ischemia
(a) Single dose administration (subgroup A)
In all hearts in subgroup A subjected to 120
minutes of ischemia, little CP remained and
lactate content increased 16-fold over the pre-
ischemic value. Tissue ATP was reduced to 40,
15 and 16 percent and tissue TAN was reduced
to 66, 52 and 52 percent of the preischemic
value in Groups I-A, II-A and III-A, respectively.
Tissue AMP was least in Group I-A and tissue

*Japanese Circulation Journal Vol. 49, January 1985*
G6P was least in Group III-A. These findings indicate that hyperkalemic cardioplegia is not advantageous in preserving high energy phosphates and calcium-free cardioplegia does not influence the myocardial metabolism (except for G6P content) under conditions of a single dose administration.

(b) Multidose administration (subgroup B)

At the end of ischemia, tissue ATP was reduced to 61, 57 and 79 percent and tissue TAN was reduced to 75, 74, and 87 percent of the pre-ischemic value in Group I-B, II-B and III-B, respectively. Statistical comparisons between three subgroups revealed that levels of ATP and TAN were best preserved in Group III-B. Lactate content was also least in Group III-B. In all groups, hearts in subgroup B resulted in a more effective preservation of high energy phosphates (I-A vs I-B p < 0.02, II-A vs II-B p < 0.001, III-A vs III-B p < 0.001) and lesser amounts of accumulating lactate (I-A vs I-B p < 0.001, II-A vs II-B p < 0.001, III-A vs III-B p < 0.001) than those in subgroup A. However, the extent of the effect of multidose administration on the myocardial metabolism clearly depended on the contents of the solution infused during ischemia.

DISCUSSION

The development of lethal injury in the ischemic myocardium is closely related to the level of tissue ATP. The delayed return of myocardial function observed after ischemia is also attributed to the prolonged depletion of ATP and adenine nucleotide pool due to its delayed re-synthesis following ischemia. These findings led to the concept that ATP and TAN levels in the ischemic myocardium are good markers for assessment of cellular integrity and recovery potential, and numerous discussions have evolved around the maintenance of ATP and total adenine nucleotide during ischemia.

It is widely accepted that hyperkalemic cardioplegia immediately arrests the heart and preserves the high energy phosphate stores by avoiding needless expenditure of these stores during ischemia. However, there have been considerable controversies over the relative importance of potassium in cardioplegic solution, under conditions of profound hypothermic ischemia. The possibility of potassium-induced cellular injury cannot be overlooked.

In the present study, when the hearts received cardioplegic solution only at the onset of ischemia (single dose cardioplegia), there was a greater decline in high energy phosphate (HEP) during ischemia in the hearts subjected to hyperkalemic cardioplegia (Group II-A, Group III-A) than in those subjected to normokalemic solution (Group I-A). Membrane depolarization initiated by elevated $[K^+]_0$ causes an increase in calcium ($Ca^{++}$) permeability and hence an increase in $Ca^{++}$ influx into the cell. It is hypothesized that more energy is needed to move $Ca^{++}$ uphill against the electromechanical gradient through active outward transport of sodium ions (Na$^+$) via the ATP-dependent Na$^+$-K$^+$ pump as long as the myocardium is depolarized. The low concentration of Na$^+$ in GIK solution which we used may also explain why HEP rapidly declined, as a reduction of extracellular sodium leads to the equilibrium described in the following equation and a shift in the ratio $[Ca^{++}]_i/[Ca^{++}]_0$ increases (0, extracellular, i, intracellular).

$$\frac{[Ca^{++}]_i}{[Ca^{++}]_0} = \frac{[Na^+]_0^2}{[Na^+]_i^5}$$

When $[Ca^{++}]_0$ is maintained constant, a decrease in $[Na^+]_0$ causes an increase in $[Ca^{++}]_i$ and more energy may be required to maintain the calcium homeostasis of the cell. The main beneficial effect of hyperkalemic cardioplegia may be to arrest immediately at the onset of ischemia when the heart cannot be cooled sufficiently to depress the myocardial metabolism. We used the isolated rat heart model as it was small enough to be cooled instantly. For this reason, the beneficial effect of hyperkalemic cardioplegia was not prominent.

Many surgeons use GIK as cardioplegic solution, on the basis of production of ATP from anaerobical glycolysis. Investigators suggested that glycolytically produced ATP is important for the maintenance of membrane function during ischemia and that glucose in combination with insulin and potassium might be beneficial for the ischemic myocardium. However, in our study, KHB produced the same dose of lactate at the end of 120 min of ischemia as did GIK. Therefore, the two approaches seem equally effective in producing high energy bonds via anaerobic glycolysis during ischemia, in the absence of washout of lactate and other toxic metabolites.

It is generally considered that multidose cardioplegia intermittently washes out the acid metabolite of anaerobic metabolism and provides
myocardial protection superior to that seen with single dose administration. Our results indicate that multidose administration of either KHB or GIK is effective in preserving HEP and that the extent of its effects varies with the composition. Walls et al. emphasized the importance of washout of products of anaerobic metabolism during ischemia, especially lactate. Our data are consistent with their findings that tissue lactate was significantly lower in multidose administration group following 120 min of ischemia.

As the concentration of free intracellular Ca\textsuperscript{2+} is of the order of 10\textsuperscript{-6} M or less and very low compared to that of extracellular levels, there is a continuous passive inflow of Ca\textsuperscript{2+} into the cell. In particular, when the myocardium is depolarized by hyperkalemic cardioplegia, there may be a greater increase in Ca\textsuperscript{2+} permeability and influx. To maintain intracellular calcium homeostasis during ischemic arrest, energy is needed to move Ca\textsuperscript{2+} uphill against the electrochemical gradient, through an energy-dependent ion transport system. Thus, if Ca\textsuperscript{2+} influx into the cell can be reduced by decreasing the gradient between [Ca\textsuperscript{2+}]\textsubscript{i} and [Ca\textsuperscript{2+}]\textsubscript{o}, the catabolism of HEP during ischemia might be depressed.

Our results show that a multidose, calcium-free infusion is beneficial for the ischemic myocardium, both to maintain HEP and to prevent lactate accumulation. However, when administered in a single dose, the beneficial effect of Ca\textsuperscript{2+}-free cardioplegia is not prominent, probably because 3 ml of Ca\textsuperscript{2+}-free cardioplegia in a single administration is too small to lower the [Ca\textsuperscript{2+}]\textsubscript{i} sufficiently to depress the Ca\textsuperscript{2+} influx into the cell. As reperfusion of isolated rat hearts with Ca\textsuperscript{2+}-containing medium, (after a Ca\textsuperscript{2+}-free perfusion) results in myocardial damage called the "calcium paradox," the use of Ca\textsuperscript{2+}-free cardioplegia during open heart surgery may be hazardous. However, there is a non-coronary collateral flow in in vivo hearts and the calcium paradox can be suppressed by decreasing the temperature of the Ca\textsuperscript{2+}-free medium. As cardioplegic solution is invariably used in combination with deep hypothermia during open heart surgery, the possibility of calcium paradox is clinically diminished.

Acknowledgments
This study was supported in part by Research Grant 57480282 from the Ministry of Education, Japan. We thank M. Ohara for comments on the manuscript and M. Sasaki and C. Yoshinaga for technical and secretarial assistance.

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Japanese Circulation Journal Vol. 49, January 1985


Japanese Circulation Journal Vol. 49, January 1983