**Functional and Metabolic Effects of Cardioplegia
Induced by a Young's Solution (YNG Solution)
as Assessed in Isolated Atrial Preparations and
in Isolated Perfused Heart Preparations of
the Guinea Pig**

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SUMMARY

The protective effects of Young's solution (YNG solution), a cardioplegic solution, on the myocardial function and metabolism were assessed in isolated atrial preparations and isolated perfused heart preparations of the guinea pig. In atrial preparations, the time to arrest of the contraction became shorter as the concentrations of the K^+ , Mg^{++} , and K^+-Mg^{++} solutions increased, while the time to resumption of the contraction was lengthened. There was no difference in the time to arrest between K^+ and K^+-Mg^{++} solutions, but the time to resumption was reduced with K^+-Mg^{++} solution to about half the value with K^+ solution. YNG solution represented an optimum solution for obtaining a quick cardiac arrest and quick resumption of contraction. In the isolated perfused heart, the time to arrest was similar for all the cardioplegic solutions used and the heart stopped in diastole. However, the time to arrest was longer with cold Krebs-Henseleit's solution (cold cardioplegia). The time to resumption of the contraction was the shortest with YNG solution, which yielded the highest mitochondrial respiratory control ratio (RCR) and ADP/O ratio. There was a negative correlation between the time to resumption of contraction and mitochondrial RCR or ADP/O ratio ($r = -0.50$ and -0.58 , respectively).

Additional Indexing Words:

Myocardial protection Cardioplegic solution Myocardial
function Mitochondrial respiratory activity Coronary flow

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IN 1955 Lam et al¹⁾ and Melrose et al²⁾ used high potassium solution to induce a quick arrest of the heart beat necessary for open heart surgery. Later, Young et al³⁾ introduced a mixture of potassium, magnesium and neostigmine, i.e. Young's solution, which resulted in a quicker recovery of the cardiac function after operation. At about the same time as the introduction of Young's solution selective cardiac hypothermia was adopted for myocardial protection. Shumway's original method⁴⁾ consisted in the topical cooling, while Mundth et al,⁵⁾ Tyers et al⁶⁾ and Stemmer et al⁷⁾ induced cardiac hypothermia by perfusion of the coronary arteries with cold Ringer solution. Recently in Japan Wada et al¹⁾ combined the two methods and have obtained excellent results in myocardial protection during operation. The cardioplegic solution they used for cold perfusion was a Young's solution without neostigmine. The present paper pertains to the critical evaluation of efficacy of the Young's (YNG) solution as a cardioplegia. The effects of changing the concentration of K^+ and Mg^{++} on the time to the cardiac arrest and the resumption of the contractions were studied in isolated atrial preparations of the guinea pig. In addition, the effect on the recovery process after cardiac arrest were studied in the isolated perfused heart preparations of the same animal in comparison with that of the cold cardioplegia and correlated with the effects on the respiratory activity of the myocardial mitochondria. Although Young et al already studied the effects of K^+ and Mg^{++} solutions on the process of cardiac arrest and recovery, the concentration ranges they tested were limited, and no attempt was made to assess the mitochondrial activities.

MATERIALS AND METHODS

1. Isolated atrial preparation

Male guinea pigs weighing between 400–600 Gm were stunned by a blow on the head. The heart was rapidly excised, atria were isolated and placed in an organ bath containing 10 ml of Krebs-Henseleit's solution maintained at a temperature of $32 \pm 0.5^\circ\text{C}$ and aerated with 95% O_2 + 5% CO_2 . One end of the atrial preparation was fixed to the bottom of the bath, while the other end was connected to a force-displacement transducer (Nihon Kohden SBIT), under a resting tension of 0.5 Gm. The tension development was recorded on an ink-writing oscillograph with a carrier amplifier (Nihon Kohden RP-5). The rate of contraction was recorded with a cardiometer (Nihon Kohden RT-5). After equilibration, the bathing solution was changed to a cardioplegic solution ($32 \pm 0.5^\circ\text{C}$) and the time to the complete arrest of the contraction was monitored. One or 5 min after the

arrest, the bathing solution was changed to Krebs-Henseleit's solution and the time to resumption of the contraction was recorded. The protocol of the experiments is depicted in Fig. 1a.

2. Isolated perfused heart preparation

a) Assessment of cardiac function

Guinea pigs of either sex weighing from 300 to 600 Gm were stunned by a blow on the head. The heart was quickly excised and retrograde perfusion through the aorta by Langendorff's method was started with modified Krebs-Henseleit's solution (NaCl, 123.9 mM; KCl, 4.7 mM; CaCl_2 , 2.5 mM; MgSO_4 , 1.2 mM; KH_2PO_4 , 1.2 mM; NaHCO_3 , 24.9 mM; glucose, 5.5 mM; sodium pyruvate, 2.0 mM) kept at a temperature of $38 \pm 0.5^\circ\text{C}$ and aerated with a gas mixture of 95% O_2 + 5% CO_2 . The perfusion pressure was set at 75 cm H_2O . A cannulating type probe of an electromagnetic flowmeter (Nihon Kohden MF 26) placed just before the aortic cannula measured the coronary inflow. The heart rate was counted with a cardiometer triggered by phasic changes of the coronary inflow.

After 1 hour's equilibration period, the perfusion solution was changed to a cardioplegic solution at $10 \pm 1^\circ\text{C}$ and the time to the arrest of contraction, the temperature of the perfusion solution and the coronary inflow at the time of the cardiac arrest were recorded. Then, the preparation was perfused for 1 hour with Krebs-Henseleit's solution ($10 \pm 1^\circ\text{C}$) under arrest of the contraction. In another group of the preparations, cardiac arrest was induced with Krebs-Henseleit's solution at $10 \pm 1^\circ\text{C}$ (cold cardioplegia). The perfusion of the preparation was continued for 1 hour after the arrest. After

Fig. 1. Experimental protocol. a) Isolated atrial preparations. b) Isolated perfused heart preparations.

1 hour's perfusion with cold Krebs-Henseleit's solution, the perfusion medium was changed to a warm Krebs-Henseleit's solution ($38 \pm 0.5^\circ\text{C}$) and the time to resumption of the contraction was recorded. The perfusion was continued for 20 min to see whether the preparation recovered regular contractions. The protocol is depicted in Fig. 1b.

b) Assessment of mitochondrial function

After 20 mins' reperfusion with Krebs-Henseleit's solution of 38°C , the heart was removed from the perfusion system and mitochondrial preparations were made following the method of Hagiwara.⁹⁾ Ventricular muscles were minced and 10 volumes of the preparation solution (mannitol, 0.21 M; sucrose, 0.07 M; EDTA-Na, 0.25 mM; Tris-HCl buffer (pH 7.4), 10 mM) precooled to a temperature below 4°C were added. Five mg/Gm wet weight of alkaline protease (crystal, 100×10^4 PUN, Nagase Sangyo) was added to this solution. After 20 mins' incubation at 0°C , the preparation was washed thoroughly with cold preparation solution and homogenized with a teflon homogenizer at 0°C . The homogenate was centrifuged at 600 g for 10 min at 0°C , and the resultant supernatant was then centrifuged at 10,000 g for 10 min. The pellet was resuspended with a small volume of cold preparation solution and centrifuged again at 600 g for 3 min. The supernatant was centrifuged at 10,000 g for 10 min. The pellet was resuspended in a reaction medium of the following composition: mannitol, 0.25 M; EDTA-Na, 0.2 mM; KCl, 10 mM; KH_2PO_4 , 10 mM; Tris-HCl buffer (pH 7.4), 10 mM. The oxygen consumption of the mitochondria was measured with a polarographic apparatus (Yanagimoto PO-100A), using *l*-glutamic acid as a substrate and ADP as a phosphate acceptor. The oxygen consumption rate of the state 3 and 4, respiratory control ratio (RCR) and ADP/O ratio were obtained to assess respiratory activity. Protein determinations were conducted following Lowry's method.¹⁰⁾

Cardioplegic solutions used in the present study were as follows:

YNG solution: 0.8% $\text{K}_3(\text{C}_6\text{H}_5\text{O}_7) \cdot \text{H}_2\text{O}$

—2.46% $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ in saline,

K^+ - Mg^{++} solution: 0.2–6.4% $\text{K}_3(\text{C}_6\text{H}_5\text{O}_7) \cdot \text{H}_2\text{O}$

—2.46% $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ in saline,

K^+ solution: 0.2–6.4% $\text{K}_3(\text{C}_6\text{H}_5\text{O}_7) \cdot \text{H}_2\text{O}$ in saline,

Mg^{++} solution: 0.62–19.7% $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ in saline.

RESULTS

1) Atrial preparation

Representative records of the changes in the atrial contractions induced

by cardioplegic solutions are shown in Fig. 2. One min after complete cessation of contractions (arrest), the solution was changed to Krebs-Henseleit's solution.

Fig. 2. Effects of YNG solution on the isolated atrial preparation. As shown in the figure, the time of the resumption of contractions was clearly discernible by a definite contraction which was followed by small, but gradually increasing contractions.

Fig. 3. Relationship between the time to arrest and ionic concentrations of cardioplegic solution in isolated guinea pig atrial preparations.

Fig. 4. Relationship between the time to resumption of the contraction and the ionic concentrations of cardioplegic solution in isolated atrial preparations, after immersion in cardioplegic solutions for 1 min to induce arrest of contractions.

As shown in Fig. 3, the time to arrest of contraction decreased as the ionic concentrations of the cardioplegic solution were increased. There was no difference between the K^+ solution and the K^+ - Mg^{++} solution, while the time to arrest was longer with the Mg^{++} -solution as compared with either the K^+ or K^+ - Mg^{++} solutions.

As shown in Fig. 4, the time to resumption of contractions in Krebs-Henseleit's solution after a 1 min incubation with cardioplegic solutions increased with increased concentrations of either solution. However, the addition of magnesium tended to decrease the resumption time by about 50%. The resumption times for K^+ - Mg^{++} solutions containing 0.4, 0.8 (YNG solution) and 1.6% K^+ were significantly shorter than for K^+ solutions of the corresponding concentrations, and the time to resumption for Mg^{++} solutions was shorter than for K^+ solutions or K^+ - Mg^{++} solutions. The same holds true for the time to resumption of the contraction after a 5 min incubation in cardioplegic solutions. After resumption of contractions, the preparation contracted regularly without abnormal contractions.

Fig. 5 depicts the relationship between the time to arrest and the time to resumption of contractions after 5 mins' incubation in cardioplegic solutions. As compared with the corresponding K^+ solutions, K^+ - Mg^{++} solutions of 0.8

Fig. 5. Relationship between the time to arrest and the time to resumption of contractions in isolated atria placed in cardioplegic solutions for 5 min to induce the arrest of contraction.

and 1.6% significantly reduced the time to resumption without changing the time to arrest of contractions. As compared with YNG solution, the time to resumption of the contraction was significantly longer with the 0.8 and 1.6% K^+ solutions or the 1.6% K^+-Mg^{++} solution, while the time to arrest was significantly longer with the 0.4% K^+ or 0.4% K^+-Mg^{++} solutions. Thus, it was possible to obtain the fastest arrest and quickest resumption of the contraction of the atrial preparations with YNG solution.

2) Isolated perfused heart

(a) Cardiac function

As listed in Table I, the time to arrest of contractions with YNG solution was 1.19 min. The time to arrest tended to be longer with the 0.4% K^+-Mg^{++} solution and the 2.46% Mg^{++} solution. The time necessary for arrest without cardioplegic solution (i.e. with cold Krebs-Henseleit's solution) was 6.07 min, a time which was significantly longer than with cardioplegic solutions. The temperature of the perfusion medium flowing out of the preparation at arrest was significantly lower with the 0.4% K^+-Mg^{++} and 2.46% Mg^{++} solutions, compared with 22°C recorded with the 1.6% K^+-Mg^{++} and

Table I. The Effects of Cardioplegic Solutions on Cardiac Function

Function	Treatment					
	0.8%K-Mg (YNG)	0.4%K-Mg	1.6%K-Mg	0.8%K	2.46%Mg	Cold car-dioplegia
Time to arrest (min)	1.19± 0.10	1.58± 0.43	0.99± 0.13	0.91± 0.13	2.56± 0.87	6.07± 1.15**
Temp. at arrest (°C)	22.0± 1.7	17.0± 0.8*	22.0± 1.3	19.8± 0.4	16.4± 0.7*	11.0± 0.4**
Max. coronary flow after arrest [% change from control ^a]	243± 16	192± 9	221± 16	215± 9	188± 37	137± 22**
Time to resumption of contractions (min)	2.20± 0.24	2.54± 0.36	3.15± 0.79	3.54± 0.48*	3.70± 0.61*	3.02± 0.29
Time to resumption of regular contractions (min)	11.78± 1.17	11.13± 1.09	10.91± 1.32	13.13± 1.54	13.22± 2.20	11.21± 0.47
Resumption of regular contractions (%)	100	60	100	80	40	80

** $p < 0.01$, * $p < 0.05$ (significant difference vs YNG).

Mean ± S.E. (n=5).

a: 5 min before cardioplegic treatment.

YNG solutions.

The temperature at arrest with cold Krebs-Henseleit's solution (11°C) was significantly lower than those with cardioplegic solutions. These findings were in agreement with the lengthening of the time necessary for the arrest of contractions. With the 0.4% K⁺-Mg⁺⁺ and 2.46% Mg⁺⁺ solutions, a sporadic resumption of contractions was seen in several instances after a temporary arrest of contractions.

The coronary flow immediately after the arrest was twice the control value in preparations arrested with cardioplegic solution, while the increase was small in preparations arrested with cold Krebs-Henseleit's solution (significantly less than those obtained with cardioplegic solution, except for the 2.46% Mg⁺⁺ solution).

The time to resumption of contractions (2.2 min) was shortest with YNG solution. It increased in the order of 0.4% K⁺-Mg⁺⁺ solution, cold cardioplegia and 1.6% K⁺-Mg⁺⁺ solution. The values obtained with 0.8% K⁺ solution (3.45 min) and 2.46% Mg⁺⁺ solution (3.70 min) were significantly larger than with YNG solution.

The time to resumption of regular contractions was slightly longer with 0.8% K⁺ and 2.46% Mg⁺⁺ solutions than with YNG solution, although the difference was not significant. Although the percentage of preparations where regular contractions resumed within 20 min was 100% with YNG solution and 1.6% K⁺-Mg⁺⁺ solution, it was 80% with 0.8% K⁺ solution and cold cardioplegia, 60% with 0.4% K⁺-Mg⁺⁺ solution and 40% with 2.46%

Mg⁺⁺ solution.

(b) Respiratory activity of the mitochondria

The respiratory control ratio (RCR) and ADP/O ratio of the mitochondrial preparations obtained from hearts arrested with cardioplegic solutions or cold Krebs-Henseleit's solution and reperfused for 20 min with Krebs-Henseleit's solution at 38°C are illustrated in Fig. 6. The values obtained with

Fig. 6. Effects of cardioplegic solutions on the mitochondrial function. Vertical lines indicate the S.E.

Fig. 7. Effects of cardioplegic solutions on the correlation between the time to resumption of contractions and the values of RCR and the ADP/O ratio.

YNG solution were significantly higher than those obtained from the hearts arrested with other cardioplegic solutions or cold Krebs-Henseleit's solution, except for the values of RCR (4.18) and ADP/O ratio (2.91) obtained from the hearts arrested with 0.4% K^+ - Mg^{++} solution and the value of RCR (4.56) obtained from the hearts arrested with cold Krebs-Henseleit's solution. These values were also lower than those with YNG solution but the differences were not significant.

(c) The relationship between the time to resumption of cardiac function and mitochondrial respiratory activity

As shown in Fig. 7, there was a significant correlation between the mitochondrial RCR and ADP/O ratio and the time to resumption of the contraction after reperfusion, indicating a dependence of the resumption of cardiac function on mitochondrial respiratory activity.

DISCUSSION

According to Young et al³⁾ and Merrit et al¹¹⁾ the ideal cardioplegic solution must satisfy the following criteria:

- (1) a quick and complete cardiac arrest,
- (2) a complete absence of electrical as well as contractile activities at the time of cardiac arrest,
- (3) minimum metabolic effects,
- (4) a quick resumption of the normal beat after reperfusion,
- (5) no ventricular fibrillation or any other serious arrhythmias,
- (6) no systemic toxicity.

Using isolated, perfused cat heart preparations, they tested the effects of several solutions on the process of cardiac arrest and recovery of regular beats and selected a mixture of 0.81% K^+ and 2.47% Mg^{2+} , i.e. Young's solution, as the best cardioplegic solution. Chiba et al¹²⁾ reported satisfactory protection of the left ventricular function and myocardial metabolism during 2 hours of aortic clamping with intermittent cold perfusion of the myocardium, using a modified Young's solution ($K_3(C_6H_5O_7) \cdot H_2O$, 0.8%; $MgSO_4 \cdot 7H_2O$, 2.46%; $NaHCO_3$, 0.021%; $NaCl$, 0.4%) as a cardioplegic solution. They concluded that the rapid production of the diastolic arrest and the resultant reduction of energy consumption produced by a modified Young's solution contributed to protection of the myocardial metabolism (and function).

The present study confirmed the superiority of YNG solution to K^+ or Mg^{++} solutions or K^+ - Mg^{++} solutions of different compositions both in the isolated heart muscle preparation and in the isolated perfused heart. Although no significant differences were observed between the isolated perfused

hearts arrested with YNG solution and those arrested with cold Krebs-Henseleit's solution with regard to either the time to resumption of contractions or the mitochondrial RCR, the time to arrest of contractions was markedly shorter and the mitochondrial ADP/O ratio was higher with YNG solution. Furthermore, the coronary flow recorded immediately after the arrest was greater with YNG solution than with the cold cardioplegia, suggesting that a more complete relaxation of the cardiac muscle was obtained with YNG solution. Thus, it may be inferred that the cardioplegia achieved with YNG solution is superior to that obtained with K^+ and Mg^{++} solutions alone, with K^+ - Mg^{++} solution of different compositions, and with cold Krebs-Henseleit's solution. This conclusion is in agreement with the findings of Ishikawa¹³⁾ that the cardiac arrest with YNG solution resulted in a better recovery than with cold cardioplegia. A better recovery with YNG solution was also reported by Kitamura¹⁴⁾ and Shimazu et al,¹⁵⁾ who ascribed it to an induction of quick diastolic arrest and a resultant better irrigation of the myocardium, which in turn brought about a quicker cooling of the myocardium.

It is conceivable that both K^+ and Mg^{++} play important roles in the protective action of YNG solution. The presence of a high concentration of K^+ is responsible for a rapid induction of diastolic cardiac arrest, while the presence of Mg^{++} inhibits myocardial metabolism.¹⁶⁾ It seems that the presence of citrate is not critical for the protection, since Merrit et al¹¹⁾ found no difference in the cardioplegic action of KCl and potassium citrate. Better preservation of mitochondrial respiratory activity may be a reflection of the protective effects of the rapid cardioplegia and the better irrigation of the myocardium. This is in agreement with a better preservation of the high energy phosphate compounds reported by several previous researchers.^{12), 17)–19)}

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