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Effect of Potassium-Channel Openers on the Release of Endothelium-Derived Hyperpolarizing Factor in Porcine Coronary Arteries Stored in Cold Hyperkalemic Solution

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Abstract: Hyperkalemic solution is widely used to protect the myocardium during open-heart surgery or to preserve donor hearts during heart or heart/lung transplants. The inhibitory effects of hyperkalemic solution on the release of endothelium-derived hyperpolarizing factor (EDHF) of coronary arteries following deep hypothermic storage (4°C) has been well studied. However, it has not been established whether potassium channel openers have protective effects on the coronary endothelial function after cold storage. This study was designed to examine this. Porcine coronary artery rings were studied in organ baths. Relaxation in response to the EDHF stimulus A23187 (nonreceptor-mediated stimulus calcium ionophore) in thromboxane A2 mimetic U46619 (30 nmol/L)-induced precontraction after incubation with hyperkalemic solution (20 mmol/L) with nicorandil (10 μmol/L) (either at 37°C in the oxygenated organ chamber or at 4°C in a refrigerator for 6 h) was compared with the control. There was significant difference between hyperkalemia group and hyperkalemia with nicorandil group under normothermia ($p = .04$). The difference was significant in the same solution between normothermia and hypothermia. After incubation in hyperkalemic solution without or with nicorandil, the A23187-induced relaxation was 32.8% ± 9.1% and 72.6% ± 16.9%, respectively ($N = 8$, $p < .01$). Potassium channel opener can attenuate the inhibitory effect of hyperkalemic solution on the release of EDHF after cold storage. **Keywords:** coronary vascular tension, hyperkalemia, normothermia, hypothermia, potassium channel opener, cardioplegia. *JECT. 2002;34:125–129*

Hyperkalemic solutions that include hyperkalemic cardioplegia and organ preservations have been widely used to protect the myocardium during open-heart surgery and heart transplantation. The effect of such solutions on coronary endothelial function has been the focus of several studies (1–5). In perfused rat hearts, previous studies have suggested that infusion of hyperkalemic cardioplegic solutions damaged coronary endothelium (1). In contrast, studies of others (2) suggested that crystalloid cardioplegia did not impair the endothelium-dependent relaxation. He and associates (6, 7) have demonstrated that endothelium-dependent relaxation mediated by the noncyclooxygenase and non-nitric oxide pathway (i.e., the endothelium-derived hyperpolarizing factor [EDHF] pathway) in porcine coronary arteries was altered by exposure to hypekalemia. Furthermore, they performed another excellent experiment and discovered that University of Wisconsin (UW) solution impaired endothelium-dependent relaxation mediated by EDHF in the coronary circulation during cold storage (8).

Endothelium plays an important modulatory role on vascular tone. Endothelium-dependent relaxation is known to be the effect of a variety of different endothelium-derived relaxing factors (EDRFs). They are endothelium-derived nitric oxide (EDNO), prostacyclin (PGI_2), and EDHF. EDNO and PGI_2 have been well studied. The nature of EDHF has not been conclusively identified, although most recently the epoxyeicosatrienoic acid (EETs) from cytochrome P450-monooxygenase metabolite of arachidonic acid has been suggested to be EDHF (9, 10). EDHF induces vascular smooth muscle relaxation via hyperpolarization of the smooth muscle cells, (11) which may involve potassium (K^+) channels, especially the opening of Ca^{2+}-activated K^+ channels (K_{Ca2^+}) (12). In contrast, EDNO and PGI_2 relax blood vessels through increasing the level of the cyclic guanisine monophosphate (cGMP) and the cyclic adenosine monophosphate (cAMP), respectively. However, all of these EDRFs are released in response to the increase of intracellular (cytosolic free) calcium concentration in the endothelial cell (7).

At concentrations that do not depress myocardial function, potassium channel openers (PCOs), such as cro-

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makalim, pinacidil, aprikalim, and nicorandil, significantly reduce the damage caused by transient global ischaemia in perfused rat heart, in vitro (13). This was shown by an improvement in the postischaemic mechanical function, an inhibition of contracture formation, a reduction in lactate dehydrogenase release, and a preservation of intracellular ATP content (14). Use of adenosine triphosphate-sensitive PCOs as cardioplegia is a potential method for cardiac protection during open-heart operations. In addition, the vasorelaxant action of PCOs may provide another important benefit to the myocardial protection. Potassium-channel openers are believed to relax blood vessels through hyperpolarization of the membrane potential of the smooth muscle. This subsequently affects voltage-operated calcium channels and intracellular calcium release, and, therefore, relaxes the vessel. In addition, our previous study demonstrated that after potassium-channel opener (PCO) nicorandil was added to hyperkalemic cardioplegia, the cardioprotective and the vasoprotective effect of nicorandil were still preserved under normothermia. However, it has not been established whether a protective effect of PCOs on coronary endothelial function, particularly EDHF-related endothelial function exists after cold storage. The present study was especially designed to examine this effect.

MATERIALS AND METHODS

Coronary arteries were obtained from porcine hearts harvested in a local abattoir. Once the pigs were sacrificed, the hearts were rapidly removed, submersed in a Krebs-filled container at 4°C, and transferred to the laboratory. Epicardial coronary arteries were dissected free from the surrounding connective tissue, cut into 3-mm long rings, and mounted on a pair of stainless steel wires in organ chambers filled by Krebs solution at 37°C. The Krebs solution had the following composition (mmol/L): Na^+ 144, K^+ 5.9, Ca^{2+} 2.5, Mg^{2+} 1.2, Cl^- 128.7, HCO_3^- 25, SO_4^{2-} 1.2, $H_2PO_4^-$ 1.2, and glucose 11. The pH of the solution is 7.4 at room temperature. The solution was aerated with a gas mixture of 95% oxygen and 5% carbon dioxide at 37°C.

An organ-chamber technique was used to normalize vascular rings under physiologic pressure with a computerized program (Powerlab Chart v3.4.3 for Windows, AD Instruments Pty Ltd, Australia).

The endothelium was intentionally preserved by cautiously dissecting and mounting the rings (7). To examine the endothelium dependence of the relaxation, in some rings the endothelium was removed mechanically by using a fine wood stick moistened with Krebs solution to rub the intima of the rings gently. This method has been shown to eliminate the endothelium-dependent relaxation in the canine coronary artery and the human internal thoracic artery. In endothelium-denuded rings, nitroglycerin (–4.5 log M) was added at the end of the experiments to test whether those rings could still be relaxed with this endothelium-independent vasorelaxant agent (7). The vascular tension development was recorded with use of the Powerlab Program (Chart v3.4.3 for Windows, AD Instruments Pty Ltd, Australia). Eight organ-chamber arrangements were run concurrently. Forty coronary artery rings were studied.

PROTOCOL

Exposure to Hyperkalemic Solution Under Normothermia

Control: All rings ($N = 40$) were equilibrated for 30 min before and after normalization. To isolate EDHF-mediated relaxation from other endothelium-derived relaxing factors, the experiments were conducted in the presence of indomethacin (7 μmol/L), a cyclooxygenase inhibitor, and N^G-nitro-L-arginine (L-NNA, 300 μmol/L), a nitric oxide synthase inhibitor for 30 min. Thromboxane A2 mimetic U46619 (30 nmol/L) was then added into the organ chamber to precontract the rings. When the contraction reached a stable plateau (usually 10 min), cumulative concentration-response curves to stimulus calcium ionophore A23187 were established. The concentrations were –10 to –6 log mol/L for A23187. The rings were then repeatedly washed, and the following protocol was applied.

Hyperkalemic Solution Exposure: When the tension of the rings returned to the previous level, the bath solution was changed to hyperkalemic solution (20 mmol/L). For the hyperkalemia group without nicorandil [N-(2-hydroxyethyl) nicotinamide nitrate] ($N = 8$) in separate experiments, rings were exposed to hyperkalemic solutions containing 20 mmol/L K^+ in Krebs' solution. To isolate the effect of hyperkalemic solution from ischemia, the hyperkalemic solution was continuously bubbled with a mixture of 95% oxygen and 5% carbon dioxide at 37°C. The incubation time to the hyperkalemic solution was 6 h. During the incubation, the tension of coronary rings was continuously recorded. After incubation for 6 h, the rings were repeatedly washed again for 30 min to restore the baseline, and L-NNA and indomethacin were added for another 30 min. The concentration-relaxation curves to A23187 were established.

Hyperkalemic and Nicorandil: In the hyperkalemia and nicorandil group ($N = 8$), the protocol was the same as in the hyperkalemia group, except that in these experiments, nicorandil (10 μmol/L) was added into the bath solution in addition to hyperkalemia.

Exposure to Hyperkalemic Solution Under Hypothermia

Cold Storage with Hyperkalemic Solution: Coronary arteries ($N = 8$) were immersed in hyperkalemic solution

(20 mmol/L) at 4°C for 6 h without oxygenation. The artery was then suspended in the organ chamber and repeatedly washed with Krebs solution. The solutions in the organ chamber were continuously aerated with a mixture of 95% oxygen and 5% carbon dioxide for the subsequent experiment. After equilibration for 1 h, the normalization procedure was performed, and the rings were equilibrated again as mentioned earlier. The relaxation to A23187 ($N = 8$) in U46619-induced precontraction was tested in the presence of indomethacin and L-NNA.

Cold Storage with Hyperkalemic and Nicorandil: After storage at 4°C for 6 h without oxygenation, the rings ($N = 8$) were the same as in the cold hyperkalemia group, except that nicorandil (10 μmol/L) was added into the bath solution in addition to hyperkalemia in these experiments.

Statistical Analysis

When the comparison was made in the same ring for the maximal response (either relaxation or contraction) under normothermia, the paired *t*-test was used. The maximal response (either relaxation or contraction) in two groups of different rings, such as in the cold storage experiments, was compared by analysis of variance (ANOVA). All statistical analysis was performed with SPPS software (SPSS, Inc, Chicago, IL).

Drugs

Drugs used and their sources were A23187, L-NNA, U46619, and indomethacin (Sigma, St. Louis, MO). Nicorandil was purchased from Japan. L-NNA (dissolved in distilled water) and indomethacin and nicorandil (dissolved in ethanol) were stored at 4°C. The solution of U46619 was held frozen until required.

RESULTS

Coronary Tension during Exposure to Hyperkalemic Solution or to Hyperkalemia Combined with Nicorandil

Under normothermia in the organ chamber, during incubation with hyperkalemic solution or hyperkalemia combined with nicorandil, the coronary tone increased to the peak of 3.97 ± 0.87 g and 2.83 ± 1.18 g in 10 minutes, respectively. There was significant difference between two groups ($p < .05$) (Table 1). Under cold storage, the coronary arteries were at resting status in a refrigerator, and there was no attempt to record the tension.

Table 1. The coronary tension after exposure to hyperkalemia under normothermia.

Groups	*N*	Latitude of Tension (g)	*p*-value
Hyperkalemia	8	2.83 ± 1.18	
Hyperkalemia with nicorandil	8	3.97 ± 0.87*	0.04

*$p < .05$.

Latitude of Precontraction of U46619

Normothermia: After exposure to hyperkalemic solution, under normothermia, the latitude of precontraction to U46619 was 3.77 ± 1.33 g in the control group, 3.11 ± 0.97 g in hyperkalemic group, and 4.17 ± 1.82 g in the hyperkalemia with nicorandil group, respectively. There was no significant difference among three groups ($p > .05$) (Table 2).

Cold Storage: After cold storage with hyperkalemic solution or hyperkalemia and nicorandil, the latitude of contraction to U46619 (30 nmol/L) did not change (1.57 ± 0.65 g vs. 1.55 ± 0.72 g, $p > .05$) (Table 2). There is significant difference in the latitude of contraction to U46619 between normothermia and hypothermia in the same group ($p < 0.05$, paired *t*-test).

Effect of Hyperkalemic Solution or Hyperkalemia Combined with Nicorandil on A23187-Induced Relaxation

Normothermia: A23187-induced relaxation was reduced from 84.24% ± 11.27% in the control group to 34.2% ± 10.85% in hyperkalemic solution exposure ($p < .001$ as compared with the control). Under hyperkalemia with nicorandil exposure, the percentage of the relaxation was 66.89% ± 18.26%, as compared with the former two groups ($p < .001$ as compared with hyperkalemic solution, and $p < .05$ as compared with the control).

Hypothermia: After hyperkalemic solution without or with nicorandil exposure, the A23187-induced relaxation was 32.75% ± 9.14% and 72.65% ± 16.92%, respectively ($p < .001$).

There is no significant difference between the control group under normothermia and hyperkalemia with nicorandil groups under hypothermia ($p > .05$).

DISCUSSION

The present study has demonstrated: (1) when added into hyperkalemic solution (20 mmol/L) under cold storage, PCO (nicorandil) may also preserve the indomethacin- and L-NNA-resistant (EDHF-related) coronary, en-

Table 2. The mean latitude of precontraction of U46619 during different situations.

Groups	*N*	Latitude of Precontraction of U46619 (g)
Normothermia		
Control	8	3.77 ± 1.33
Hyperkalemia	8	3.11 ± 0.97
Hyperkalemia with nicorandil	8	4.17 ± 1.82
Hypothermia		
Hyperkalemia	8	1.57 ± 0.65*
Hyperkalemia with nicorandil	8	1.55 ± 0.72†

*Differences between normothermia and hypothermia incubation with hyperkalemic solution. *$p < .05$.
†Difference between normothermia and hypothermia incubation with hyperkalemia with nicorandil. †$p < .05$.

Table 3. The effect of hyperkalemic solution or hypokalemia combined with nicorandil on A23187-induced relaxation.

Groups	*N*	A23187-Induced Relaxation (%)	*p*-value
Normothermia			
Control	8	84.24 ± 11.27	
Hyperkalemia	8	34.20 ± 10.85***	0.001
Hyperkalemia with nicorandil	8	66.89 ± 18.26*††	0.001
Hypothermia			
Hyperkalemia	8	32.75 ± 9.14	
Hyperkalemia with nicorandil	8	72.65 ± 16.92**	0.001

*Compared with the control during normothermia. *$p < .05$. ***$p < .01$.
†Difference between hyperkalemia without and with nicorandil during normothermia. ††$p < .01$.
**Difference between hyperkalemia without and with nicorandil during hypothermia. **$p < .01$.

dothelium relaxation function, as it is used under normothermia; and (2) under the same situation as above, PCO (nicorandil) has no influence on contractility of coronary artery to U46619. However, the coronary smooth muscle contractility is protected by hypothermia.

The findings from the present study, in addition to our previous ones (unpublished), may reveal a mechanism for endothelial dysfunction after exposure to hyperkalemic solution (20 mmol/L) and, therefore, have clinical implications in transplantation surgery and cardiac surgery.

Coronary Endothelial Function

Endothelial cells derive different substance of relaxation and contraction. Endothelium-dependent relaxation is known to be caused by a variety of EDRFs. These are EDNO, PGI_2, and EDHF. Unlike EDNO and PGI_2, which have been well studied, the nature of EDHF has not been finally identified; although, most recently, the cytochrome P450-monooxygenase metabolite of arachidonic acid has been suggested to be EDHF (9, 10). EDHF induces vascular smooth muscle relaxation via hyperpolarization of the smooth muscle cells, (11) which may involve potassium (K^+) channels (12). In contrast, EDNO relaxes blood vessels through the cyclic guanosine monophosphate pathway. However, all of these EDRFs are released in response to the increase of intracellular (cytosolic) free calcium concentration in the endothelial cell (12).

Although the exact role of EDHF in regulating vascular tone and the development of vascular diseases is still unclear, there is evidence that EDNO and EDHF are two primary mechanisms of endothelium-dependent relaxation (15). In the present study, the EDHF stimuli A23187 induced 84.7% relaxation. This suggested the role of EDHF in regulating the coronary tone at least under the stimulated condition when the PGI_2 and EDNO mechanism are blocked. Studies also show that EDHF may back up or enhance the relaxing action of EDNO, particularly when EDNO-mediated relaxation is impaired, (15) as seen in some pathologic states, such as hypercholesterolemia, hypertension, and diabetes mellitus (15). It may be true that in the coronary circulation during ischemia and reperfusion period, when the EDNO mechanism is impaired, the EDHF mechanism may play an important role in regulating the coronary circulation and developing vasculopathy (16).

Coronary Tension During Exposure to Hyperkalemic Solution or to Hyperkalemia Combined with Nicorandil

K^+ is a depolarizing agent and a strong vasoconstrictor. There is concern that hyperkalemia may constrict vessels during the exposure (preservation) period. Our present study also provides evidence that the contraction of the coronary artery is not apparently different after the incubation with hyperkalemic solution and hyperkalemia combined with nicorandil.

EDHF-Related Relaxation after Hyperkalemic Solution or to Hyperkalemia Combined with Nicorandil Exposure

The effect of hyperkalemia on EDHF-mediated endothelial function is shown by the reduction of the relaxation induced by A23187 after exposure to hyperkalemic solution for 6 h under both normothermia and hypothermia. In the normothermia experiments, the A23187-induced relaxation was reduced to 34.2% in the hyperkalemia group only ($p < .001$), 66.89% in hyperkalemia with nicorandil group ($p < .05$), respectively. What is the effect of hypothermic storage on EDHF-related relaxation? Clinically, hyperkalemic solution is designed for cold storage of the organ. It has been shown that temperature is an important factor when UW solution is used for the preservation of the rat heart (17). It has not been established whether impaired EDHF-mediated relaxation occurs during hypothermic and hypoxic storage, especially whether a protective effect of PCOs exists after cold storage under the same situation. Our study was specially designed to examine this effect. The coronary artery was stored in cold hyperkalemic solution (4°C) for 6 h without oxygenation, a situation similar to the clinical setting. We also studied the other method of storage similar to the former except nicorandil added. Our results clearly demonstrated that EDHF-mediated relaxation was impaired regardless of cold storage with hyperkalemic solution only or after exposure to K^+ for 6 h under normothermia, while it is preserved when nicorandil was added. Therefore we believe that the influence of hyperkalemic solution on the coronary EDHF-mediated relaxation does exist in the clinical settings when the heart is arrested by hyperkalemia cardioplegia or the donor organ is stored in cold UW.

Possible Mechanism of Impairment of the Relaxation and Preservation of PCOs

Some studies (8) have demonstrated that there are two mechanisms related to reduced EDHF-mediated relax-

ation after exposure to hyperkalemia. First, it is attributable to the prolonged membrane hyperpolarization of the smooth muscle. Second, exposure to hyperkalemia affects K^+ channels (particularly calcium-activated K^+ channels, which is related to the EDHF-mediated hyperpolarization in the coronary artery).

Because hyperpolarizing cardioplegia is still at an experimental stage, the cardioprotective mechanism of the PCOs remains unclear. The prevailing explanation is that PCOs can preserve EDHF-mediated endothelial function that may be damaged by hyperkalemia.

Contractility of Coronary Artery after Exposure to Hyperkalemia

Under normothermia, after exposure to hyperkalemia, the contraction to U46619 was reduced to 3.11 ± 0.97 gm as compared with 4.17 ± 1.82 gm when nicorandil was added ($p > .05$). This suggests that the coronary artery is still at a partially plegic status immediately after exposure to either hyperkalemia or hyperkalemia with nicorandil. However, the contractility of the smooth muscle was preserved after cold storage with hyperkalemia (1.57 ± 0.65 gm) and with nicorandil (1.55 ± 0.72 gm, $p > .05$). This is shown by the result that the contraction by U46619 (30 nmol/L) after cold storage with hyperkalemia or hyperkalemia with nicorandil was similar to that in the control group.

In summary, exposure to hyperkalemia at either 37°C or 4°C impairs the EDHF-mediated endothelium-dependent relaxation, although cold storage with hyperkalemia may protect the contractility of the coronary smooth muscle. After cold storage, PCO can attenuate inhibitory effect of hyperkalemic solution on the release of EDHF.

Clinical Implications

The present study suggests that during incubation with hyperkalemia the coronary tone is reduced and, therefore, no vasoconstrictor effect is seen in this period. However, during reperfusion (after incubation) the EDHF-mediated relaxation is significantly impaired. The impairment of the EDHF-mediated relaxation reduces the functional reserve of the endothelium-dependent relaxation and, therefore, may impair myocardial perfusion.

In addition, because endothelium also plays an important role in antiplatelet aggregation and prevention of atherosclerosis, the endothelial dysfunction observed in the present study may have an adverse effect on the long-term results of heart or other organ transplantation. In fact, in cardiac allografts preserved in hyperkalemia, the incidence of late graft atherosclerosis has been twice as high as grafts preserved in Stanford solution.

The findings from the present study may provide some insight into the cause of higher atherosclerotic incidence after heart transplantation using hyperkalemia and open a new area in terms of better preservation of coronary or other vascular endothelium for organ transplantation by adjusting components, such as adding PCOs. However, the impact of impaired EDHF on the development of post-transplantation atherosclerosis requires further study.

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