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·ORIGINAL ARTICLES·

Role of Nitric Oxide in the Cardioprotection of Heat Stress-Induced Delayed Preconditioning in Rats

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Aim To study the role of nitric oxide (NO) in the preconditioning of the heart with hyperthemia in rats.

Methods The isolated rat heart was perfused in a Langendorff model. Hearts for all groups were subjected to 4 h hypothermia (4 ℃) and 40 min reperfusion. Heat stress was induced by whole-body hyperthermia (rectal 42 ℃, 15 min) 24 h before the experiment. Heart rate, coronary flow, left ventricular pressure, and its derivative ($\pm dp/dt_{max}$) were recorded, and tumor necrosis factor-alpha (TNF-α) immunoreactivity in myocardial tissues and the activity of creatine kinase (CK) in the coronary effluent were measured.

Results Pretreatment with hyperthermia significantly improved the recovery of cardiac protection, reduced the release of CK, and increased plasma concentrations of NO. Pretreatment with L-NAME, an inhibitor of NOS, abolished the protective effects and the increased level of TNF-α and NO elicited by hyperthermia.

Conclusion Endogenous NO is involved in the cardioprotection afforded by heat stress, and the beneficial effects of NO are related with stimulation of TNF-α production in the rat heart.

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1 INTRODUCTION

Many methods have been used to strengthen the protective effects of St Thomas solution in the storage of heart transplant and cardiac-bypass surgery. Recently, it has been reported that ischemia-, hypoxia-, drug- or hyperthermia-induced preconditioning protects against myocardial damages after prolonged cardioplegic arrest[1]. The mechanism responsible for preconditioning is not fully understood. Amounting evidence suggests that endogenous chemical substances may play a key role in the mediation of protective effects of preconditioning induced by the above factor described. It has been shown that nitric oxide (NO) is involved in the mediation of ischemic or pharmacological preconditioning[2,3]. There is evidence to suggest that hyperthermia induces NO production[4]. It is not know, however, whether endogenous NO also participates in the mediation of hyperthermia-induced preconditioning.

It has been shown that tumor necrosis factor-alpha (TNF-α) possesses numerous pathophysiological actions. Recently, it has been reported that TNF-α may be beneficial to the ischemic myocardium in heat stress[5]. In the present study, therefore, we examined whether NO-mediated delayed preconditioning by hyperthermia is related to TNF-α production.

2 MATERIALS AND METHODS

2.1 Reagents

L-nitroarginine methyl ester (L-NAME) was purchased from the Sigma (St louis, MO. USA). Radioimmunoassay kits for measurement of TNF-α were obtained from Dongya Immunity Institute (Beijing, China). Creatine kinase assay kits were obtained from Zhongshen Bioengineering Co (Beijing, China).

2.2 Preparation of the isolated heart

Male Sprague-Dawley rats weighting 180-220 g were obtained from Hunan Medical University Animal Center. Animals were anaesthetized with sodium pentobarbital (60 mg/kg, ip). The heart was excised rapidly into Kreb-Henseleit (K-H) buffer solution at 4 ℃, and then perfused retrogradly in a non-recirculating system in a Langendorff model, at constant perfusion pressure of 100 cm

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H_2O. The heart was perfused with K-H buffer (pH 7.4), saturated with 95% O_2 and 5% CO_2. The K-H buffer had the following composition (mmol/L): NaCl 119.0, $NaHCO_3$ 25.5, KCl 4.3, KH_2PO_4 1.2, $MgSO_4$ 1.2, $CaCl_2$ 2.5 and glucose 11.0.

A water-filled latex balloon was inserted into the left ventricular and adjusted to a left-ventricular (LV) end-diastolic pressure of 3-4 mm Hg. The left ventricular pressure, its derivatives ($\pm dp/dt$) and heart rate were monitored continously. The resulting electrical signals were digitized by a Maclab analogue-to-digital converter and recorded on a Power Macintosh 7200 computer. Coronary flow was measured by timed collection of the coronary effluent and samples of coronary effluent at 5 min of reperfusion were collected for measurement of creatine kinase.

2.3 Creatine kinase assay

Myocardial injury was monitored by assaying creatine kinase (CK) released from the heart. The activity of CK in the coronary effluent at 5 min of reperfusion was measured spectrophotometrically.

2.4 Determination of plasma NO concentration

Blood samples (3 mL) were collected from carotid artery into tubes containing 10% Na_2EDTA 30 μL. The plasma was obtained by centrifugation at 3 000 r/min for 20 min at 4 ℃. The plasma level of NO was measured indirectly by the content of nitrite and nitrate spectrophotometrically.

2.5 Measurement of myocardial TNF-α

At the end of the experiment, the left ventricular myocardium of every heart was excised and added to its 10-fold volume of cold isotonic homogenized buffer (mmol/L, PMSF 1.0, KH_2PO_4 13.4 and K_2HPO_4 86.6, pH 7.6).

The individual tissue sample was homogenized with a vertishear tissue homogenizer at half-maximal speed for 20 s (10 equally spaced bursts) followed by centrifugation at 5 000 r/min for 15 min at 4 ℃. The supernatant was collected to store at − 70 ℃ until assay.

The cardiac content of TNF-α was determined by radioimmunoassay kits using antisera raised against rat TNF-α, ^{125}I-labelled TNF-α and rat TNF-α standard.

2.6 Experimental protocols

Twenty-four animals were randomly divided to four groups. For the studies on the effects of hyperthermia on the preservation with cardioplegia, the control group and the hyperthermia group were placed. In the hyperthermia-treated group, the rat was pretreated with whole-body hyperthermia (rectal 42 ℃, 15 min) 24 h before the experiment. In the L-NAME group, the rats were administrated with L-NAME (10 mg/kg, ip). And in the L-NAME plus hyperthermia-treated group, for the studies on the effect of L-NAME on protective effects of heat stress, the rats were treated with hyperthermia after pretreatment with L-NAME for 30 min. All hearts had an initial stabilization period for 20 min, and then infused with St. Thomas cardioplegia solution (4 ℃) for 2 min through a sidearm of the cannula. The St Thomas cardioplegia solution had the following composition (mmol/L): NaCl 110, KCl 16, $MgCl_2$ 16, $CaCl_2$ 1.2, and $NaHCO_3$ 10. The isolated hearts were immersed in cardioplegic solution, maintained at 4 ℃ for 4 h, and then reperfused with K-H solution for 40 min.

2.7 Statistical analysis

All values are expressed as $\bar{x} \pm s$. Statistical analysis was carried out by analysis of variance and the Newman-Keuls test. The level of significance was chosen as $P < 0.05$.

3 RESULTS

There were no significant differences in the basic values of left ventricular pressure and $\pm dp/dt_{max}$, coronary flow and heart rate before hypothermic ischemia. A decline in cardiac function (left ventricular pressure, $\pm dp/dt_{max}$, coronary flow and heart rate) and an increase in the release of CK were shown during reperfusion after 4 h of ischemia. Pretreatment with hyperthermia caused a significant improvement of cardiac function and a decrease in the release of CK. The protective effects of heat stress were abolished by L-NAME, an inhibitor of inducible NO synthase (Table 1 and 2).

Heat stress induced by pretreatment with whole body hyperthermia for 15 min produced a significant increase in the cardiac content of TNF-α. The increased level of TNF-α induced by hyperthermia was abolished by pretreatment with L-NAME (Table 2).

4 DISSCUSION

The results of the present study confirm our recent observations that delayed preconditioning induced by hyperthermia enhances preservation with cardioplegia, as shown by improvement of the recovery of cardiac function and reduction of creatine kinase release[6]. Recently, it has been found that hyperthermia-induced early preconditioning is also capable of improving preservation with cardioplegia[1]. These results suggest that heat stress-in duced preconditioning, early or delayed, improves preservation with cardioplegia.

As mentioned above, endogenous active mediators including NO play a pivotal role in the protective effects of ischemic preconditioning[3]. Others have shown that

Table 1. Effect of heat stress on cardiac function

Groups	n	Preischemia	Reperfusion (min)				
			5	10	20	30	40
			Left ventricular pressure (kPa)				
Ischemia/reperfusion	6	15.8±2.9	6.5±1.3	6.8±0.7	7.7±1.6	8.2±1.3	8.2±1.3
+ Heat stress (HS)	6	15.2±2.1	11.6±1.2[c]	12.1±0.9[c]	12.4±1.3[c]	12.8±1.1[c]	12.8±1.1[c]
+ L-NAME	6	16.4±2.9	7.8±1.9[a]	8.0±1.9[a]	7.6±1.6[a]	7.3±0.7[a]	7.3±1.5[a]
+ L-NAME & HS	6	15.4±4.5	7.4±1.6[f]	7.8±1.5[f]	7.8±2.1[f]	7.4±2.1[f]	7.4±2.1[f]
			+ dp/dt_{max} (kPa/s)				
Ischemia/reperfusion	6	555±134	201±66	204±79	251±67	259±47	265±47
+ Heat stress (HS)	6	540±41	360±51[c]	381±47[c]	399±60[c]	434±59[c]	425±59[c]
+ L-NAME	6	552±92	249±64[a]	255±64[a]	256±66[a]	259±46[a]	256±46[a]
+ L-NAME & HS	6	533±148	225±54[f]	247±60[f]	266±74[f]	266±86[f]	260±82[f]
			- dp/dt_{max} (kPa/s)				
Ischemia/reperfusion	6	395±109	154±57	152±62	177±70	169±36	180±43
+ Heat stress (HS)	6	375±40	245±29[c]	264±23[c]	282±35[c]	304±28[c]	298±27[c]
+ L-NAME	6	404±48	190±71[a]	156±34[a]	177±39[a]	172±31[a]	170±34[a]
+ L-NAME & HS	6	375±121	152±28[f]	165±36[f]	170±49[f]	177±62[f]	180±68[f]
			Coronary flow (mL/min)				
Ischemia/reperfusion	6	10.4±1.1	6.7±0.6	6.6±0.8	6.7±0.8	6.8±0.7	6.5±1.0
+ Heat stress (HS)	6	10.9±1.6	10.4±1.6[c]	10.5±1.7[c]	10.4±1.5[c]	10.5±1.7[c]	10.5±1.7[c]
+ L-NAME	6	11.0±2.3	6.5±1.5[a]	6.4±1.5[a]	6.6±1.6[a]	6.7±1.6[a]	6.2±1.5[a]
+ L-NAME & HS	6	1.4±2.4	7.0±0.8[f]	6.8±0.9[f]	7.1±0.7[f]	6.9±0.6[f]	6.7±0.9[f]
			Heart rate (beats/min)				
Ischemia/reperfusion	6	287±17	264±50	258±57	259±49	272±51	256±51
+ Heat stress (HS)	6	306±26	320±52[a]	295±21[a]	292±25[a]	290±11[a]	283±14[a]
+ L-NAME	6	296±24	262±20	275±36	296±25	295±21	294±22
+ L-NAME & HS	6	326±41	265±71[d]	262±68[d]	262±72[d]	272±78[d]	269±86[d]

Values are expressed as $\bar{x}\pm s$. [a] $P>0.05$, [c] $P<0.01$ vs ischemia/reperfusion, [d] $P>0.05$, [f] $P<0.01$ vs HS. HS: Heat stress; L-NAME: L-nitroarginine methyl ester.

Table 2. Effect of Heat Stress on the activity of creatine kinase (CK) in coronary effluent, the serum level of NO, and the cardiac content of TNF-α.

Groups	*n*	CK [u/(min•g)(wet wt)]	NO (mg/L)	TNF-α (μg/g)
Ischemia/reperfusion	6	0.94±0.18	23±10	120±22
+ Heat stress (HS)	6	0.38±0.14[c]	89±20[c]	538±78[c]
+ L-NAME	6	0.89±0.24[a]	35±10[a]	134±29[a]
+ L-NAME & HS	6	0.97±0.21[f]	39±13[f]	155±28[f]

Values are expressed as $\bar{x}\pm s$. [a] $P>0.05$, [c] $P<0.01$ vs ischemia/reperfusion, [f] $P<0.01$ vs HS. HS: Heat stress; L-NAME: L-nitroarginine methyl ester.

monophosphoryl lipid A-induced preconditioning is also related to stimulation of NO production[7]. Recently, administration of nitroglycerin, a donor NO, induces a preconditioning-like preconditioning[2]. Based on the production of NO stimulated by hyperthermia, we hypothesize that endogenous NO may be involved in the mediation of the protection afforded by heat stress. The present results revealed that a brief period of hyperthermia (15 min) caused a significant increase in the plasma level of NO concomitantly with an improvement of the recovery of cardiac function, and pretreatment with L-NAME, a inhibitor of NO synthase, abolished the elevated increase of NO and protective effects induced by hyperthermia. These results suggest that NO is a common mediator of preconditioning induced by a variety of factors including ischemia, drugs, and heat stress.

TNF-α, a cytokine, has complex cardiovascular actions[8]. Previous investigations have suggested that TNF-α may contribute myocardial damages during ischemia-rep-

efusion[9]. However, in the present study the elevated level of TNF-α induced by hyperthermia showed a protection of the ischemic myocardium. Recently, it has been reported that ischemia-induced apoptosis of myocytes in the mice lacking TNF-receptors is exacerbated compared to the wild-type mice (TNF receptors persistent)[10]. Others have also found that the cardioprotection induced by hyperthermia is abrogated by TNF-α antibody[5]. These results suggest that TNF-α is beneficial to the ischemic myocardium in heat stress.

The biosynthesis of TNF is modulated by multiple factors, and NO, endogenous or exogenous, has been shown to induce TNF-α production[11,12]. In the present study, pretreatment with hyperthermia caused a marked increase in the level of NO and TNF-α. However, pretreatment with L-NAME abolished the increased level of TNF-α and the protection induced by hyperthermia. These results support the hypothesis that NO-mediated delayed preconditioning by heat stress is related to stimulation of TNF-α production.

In summary, the present study suggests that endogenous NO is involved in the cardioprotection afforded by heat stress, and that the beneficial effect of NO is related to the stimulation of TNF-α production.

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一氧化氮在大鼠热应激诱导延迟心脏保护中的作用

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[**主题词**] 缺血—再灌注损伤; 肿瘤坏死因子; 热应激; 一氧化氮

目的 研究一氧化氮在热应激增强停搏液心脏保护中的作用。**方法** 采用 Lengendorff 装置灌注离体心脏。心脏低温(4 ℃)保存 4 h 后, 再灌注 40 min (37 ℃)。实验前 24 h 大鼠进行高温处理(直肠温度 42 ℃, 15 min)。记录心率, 冠脉流量、左室内压以及最大变化速率, 并测定血浆一氧化氮(NO)浓度和冠脉流出液中肌酸激酶(CK)释放量, 心肌组织肿瘤坏死因子 (TNF-α) 浓度。**结果** 热应激能显著增强停搏液的心脏保护作用, 减少肌酸激酶释放量, 并升高血浆一氧化氮及心肌组织肿瘤坏死因子 α 浓度。这些作用能被预先给予亚硝基精氨酸甲酯所取消。**结论** 一氧化氮参与对大鼠心脏热应激诱导的延迟保护, 其作用与促进肿瘤坏死因子的产生有关。

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