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Monophosphoryl lipid A-induced delayed preconditioning is mediated by calcitonin gene-related peptide

Shang-You He, Han-Wu Deng, Yuan-Jian Li *

Department of Pharmacology, Hunan Medical University, Changsha, Hunan 410078, People's Republic of China

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Abstract

The delayed preconditioning of the heart by monophosphoryl lipid A is mediated by endogenous nitric oxide (NO), and the cardioprotection afforded by nitroglycerin is related to stimulation of calcitonin gene-related peptide (CGRP) release. The objective of this study was to explore whether improvement of preservation with cardioplegia by monophosphoryl lipid A is mediated by CGRP. In addition, we examined the effect of monophosphoryl lipid A on the tumor necrosis factor-α (TNF-α) content of myocardial tissues. The isolated rat heart was perfused in the Langendorff mode. Heart rate, coronary flow, left-ventricular pressure, and its first derivatives ($\pm dp/dt_{max}$) were recorded, and plasma levels of NO and CGRP, the release of creatine kinase in coronary effluent and the content of TNF-α in myocardial tissues were measured. Hypothermic ischemia for 4 h caused a decline in cardiac function, and an increase in the release of creatine kinase and in the content of TNF-α. Pretreatment with monophosphoryl lipid A (500 μg/kg, i.p.) for 24 h improved the recovery of cardiac function and reduced the release of creatine kinase concomitantly with a decrease in the content of cardiac TNF-α. Monophosphoryl lipid A markedly increased plasma concentrations of CGRP and NO. After pretreatment with L-nitroarginine methyl ester (L-NAME), the cardioprotection and the increased release of NO and CGRP induced by monophosphoryl lipid A were abolished. Capsaicin also abolished the cardioprotection and the increased release of CGRP induced by monophosphoryl lipid A, but did not affect the content of NO. The results suggest that monophosphoryl lipid A-induced preconditioning enhances preservation with cardioplegia and that the protective effects of monophosphoryl lipid A are related to stimulation of CGRP release.

Keywords: Preconditioning; CGRP (calcitonin gene-related peptide); TNF-α (tumor necrosis factor-alpha); NO (nitric oxide); Heart, rat

1. Introduction

Since the term ischemic preconditioning was introduced by Murry et al. (1986), considerable progress has been made in understanding this phenomenon. It had been reported that ischemic stimulus not only triggers early preconditioning, but also induces delayed protection or "second window protection" (Yellon et al., 1998). Evidence suggests that substitution of some drugs for ischemic stimulus is also capable of inducing similar protection of ischemic preconditioning, termed pharmacological preconditioning. It has recently been shown that ischemia-, hypoxia-, or drug-induced early preconditioning enhances preservation with cardioplegia (Lu et al., 1996; Engelman et al., 1995; Thourani et al., 1999). Mounting evidence has suggested that endogenous active substances including neurotransmitters and autacoids are involved in the mediation of ischemic or pharmacological preconditioning. Monophosphoryl lipid A-induced delayed preconditioning is ascribed to stimulation of nitric oxide (NO) production (Zhao et al., 1997).

Calcitonin gene-related peptide (CGRP), a principal transmitter in capsaicin-sensitive sensory nerves, has been shown to participate in the mediation of ischemic preconditioning or heat stress (Ferdinandy et al., 1997; Lu et al., 1999; Song et al., 1999a). Recently, it has been reported that the cardioprotection afforded by nitroglycerin, a NO donor, is also related to stimulation of CGRP release (Hu et al., 1999). It has been shown that CGRP also participates in the mediation of delayed preconditioning (Zhou et al., 1999). In view of the involvement of endogenous NO in monophosphoryl lipid A-induced delayed preconditioning and the release of CGRP stimulated by NO, we postulate that endogenous CGRP may be involved in the delayed protection afforded by monophosphoryl lipid A.

* Corresponding author. Tel.: +86-731-4805441; fax: +86-731-4471339.

E-mail address: liyj@public.cs.hn.cn (Y.-J. Li).

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Tumor necrosis factor-α (TNF-α) has been recognized as a contributor to myocardial damage during ischemia–reperfusion. More recently, it has been found that ischemic preconditioning significantly decreases the content of TNF-α in myocardial tissues, and one hypothesizes that TNF-α may be an ultimate effector mechanism in ischemic preconditioning (Meldrum et al., 1998). There is evidence that CGRP inhibits the generation of TNF-α (Millet and Vignery, 1997; Feng et al., 1997). It is probable that CGRP-mediated preconditioning protects against myocardial injury through reduction of TNF-α content.

The purpose of the present study was threefold. First, we tested whether monophosphoryl lipid A-induced delayed preconditioning improves preservation with cardioplegia. Secondly, we investigated the possible contribution of endogenous CGRP in the cardioprotection afforded by monophosphoryl lipid A. Thirdly, the influence of monophosphoryl lipid A-induced preconditioning on the cardiac TNF-α content was examined.

2. Methods and materials

Male Sprague–Dawley rats weighing 180–220 g were obtained from the Hunan Medical University Animal Center. All animals received humane care in compliance with "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 National Academy of Sciences and Published by the National Institute of Health (NIH Publication no. 85-23, revised 1985).

2.1. Perfusion technique

The animals were anaesthetized with sodium pentobarbital (60 mg/kg, i.p.) and the heart was excised rapidly into Krebs–Henseleit (K–H) buffer solution at 4°C. The hearts were then perfused retrogradely in the Langendorff mode, at a constant perfusion pressure of 100 cm H_2O. The hearts were 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 ventricle and adjusted to a left-ventricular end-diastolic pressure of 3 to 4 mm Hg. The left-ventricular pressure, its first derivatives ($\pm dp/dt_{max}$) and heart rate were monitored continuously. The resulting electrical signals were digitized with 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.

2.2. Experimental protocols

Thirty-six animals were randomly divided into six groups. (1) The monophosphoryl lipid A group was injected with monophosphoryl lipid A (500 μg/kg, dissolved in 0.2% triethylamine), intraperitoneally 24 h before the experiment. (2) The monophosphoryl lipid A vehicle group was injected intraperitoneally with monophosphoryl lipid A vehicle 24 h before the experiment. (3) The L-NAME group was injected with L-NAME (10 mg/kg i.p.) 24 h before the experiment. (4) The monophosphoryl lipid A plus L-NAME group was injected simultaneously with monophosphoryl lipid A and L-NAME intraperitoneally 24 h before the experiment. (5) The capsaicin plus monophosphoryl lipid A group was injected with capsaicin (50 mg/kg s.c.) 4 days before the experiment, and then injected with monophosphoryl lipid A (500 μg/kg i.p.) 24 h before the experiment. Capsaicin (dissolved in a vehicle containing 10% Tween 80, 10% ethanol and 80% saline) was injected subcutaneously under sodium pentobarbital anesthesia. (6) The capsaicin vehicle plus monophosphoryl lipid A group was injected with capsaicin vehicle and monophosphoryl lipid A according to the same procedure as for the capsaicin plus monophosphoryl lipid A group.

The hearts from all groups were equilibrated for 20 min, and then infused with St. Thomas cardioplegia solution (4°C) 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 cardioplegia solution, maintained at 4°C for 4 h, and then were reperfused with K–H solution for 40 min.

2.3. Creatine kinase assay

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

2.4. Determination of plasma CGRP concentration

Blood samples (3 ml) were collected from the carotid artery into tubes containing 10% Na_2EDTA 30 μl and aprotinin 400 mU/l. The plasma was obtained by centrifugation at 3000 rpm for 20 min at 4°C. CGRP-like immunoreactivity in the plasma was measured using antisera raised against rat CGRP, ^{125}I-labelled CGRP and rat CGRP standard (Tang et al., 1997).

2.5. Measurement of plasma NO concentration

Blood samples were collected in the same way as above except that aprotinin was added. The plasma level of NO was measured indirectly as the content of nitrite and nitrate estimated spectrophotometrically.

2.6. Measurement of myocardial TNF-α

At the end of the experiment, the left-ventricular myocardium of every heart was excised and added to a 10-fold volume of cold isotonic homogenized buffer (mmol/l phenylmethyl sulfonyl fluoride, 1.0; KH_2PO_4, 13.4 and K_2HPO_4, 86.6; pH 7.6). Individual tissue samples were homogenized with a tissue homogenizer at half-maximal speed for 20 s (10 equally spaced bursts) followed by centrifugation at 5000 rpm for 15 min at 4°C. The supernatant was collected and stored at −70°C until assay.

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

2.7. Reagents

Capsaicin, MLA and L-nitroarginine methyl ester (L-NAME) were purchased from Sigma (St. Louis, MO, USA). Radioimmunoassay kits for measurement of CGRP and TNF-α were obtained from the Immunity Institute of Dongya (Beijing, P.R. China). Creatine kinase assay kits were obtained from Zhongsheng Bioengineering. NO assay

Table 1
Effect of monophosphoryl lipid A on cardiac function
Values are means ± S.E.M., $n = 6$.

	n	Pre-ischemia	Reperfusion (min)				
			5	10	20	30	40
Left-ventricular pressure (mm Hg)							
Vehicle (MLA)	6	87 ± 7	42 ± 4	42 ± 4	42 ± 3	42 ± 4	43 ± 5
MLA	6	86 ± 2	66 ± 5[a]	66 ± 4[a]	70 ± 2[a]	71 ± 0.8[a]	72 ± 2[a]
L-NAME	6	94 ± 9	45 ± 4	46 ± 4	47 ± 4	46 ± 4	43 ± 4
L-NAME and MLA	6	81 ± 11	41 ± 6[b]	40 ± 6[b]	40 ± 6[b]	38 ± 7[b]	40 ± 7[b]
Vehicle (Cap) and MLA	6	80 ± 5	65 ± 4	64 ± 3	64 ± 3	69 ± 5	68 ± 5
Cap and MLA	6	85 ± 11	39 ± 2[c]	40 ± 2[c]	40 ± 2[c]	44 ± 3[c]	44 ± 3[c]
$+dp/dt_{max}$ *(mm Hg/s)*							
Vehicle (MLA)	6	2588 ± 138	1090 ± 126	1206 ± 127	1221 ± 134	1250 ± 146	1282 ± 174
MLA	6	2871 ± 92	2118 ± 173[a]	2102 ± 114[a]	2327 ± 96[a]	2356 ± 116[a]	2344 ± 88[a]
L-NAME	6	2793 ± 245	1243 ± 148	1208 ± 142	1298 ± 146	1287 ± 143	1264 ± 124
L-NAME and MLA	6	2515 ± 276	1211 ± 121[b]	1159 ± 117[b]	1072 ± 145[b]	1051 ± 174[b]	1109 ± 163[b]
Vehicle (Cap) and MLA	6	2515 ± 191	2245 ± 288	2005 ± 259	2330 ± 234	2352 ± 230	2412 ± 255
Cap and MLA	6	2763 ± 214	1107 ± 120[c]	1143 ± 131[c]	1259 ± 149[c]	1329 ± 157[c]	1265 ± 168[c]
$+dp/dt_{max}$ *(mm Hg/s)*							
Vehicle (MLA)	6	2261 ± 204	924 ± 111	961 ± 87	985 ± 72	988 ± 88	1026 ± 130
MLA	6	2427 ± 97	1666 ± 152[a]	1708 ± 97[a]	1863 ± 82[a]	1889 ± 76[a]	1928 ± 78[a]
L-NAME	6	2128 ± 182	1002 ± 169	1127 ± 192	1040 ± 148	1004 ± 126	1064 ± 122
L-NAME and MLA	6	2282 ± 198	904 ± 68[b]	876 ± 93[b]	876 ± 95[b]	835 ± 99[b]	919 ± 95[b]
Vehicle (Cap) and MLA	6	2487 ± 228	1562 ± 102	1863 ± 217	1875 ± 206	1979 ± 161	1889 ± 203
Cap and MLA	6	2319 ± 264	921 ± 111[c]	963 ± 118[c]	1040 ± 153[c]	1052 ± 173[c]	1034 ± 186[c]
Coronary flow (ml/min)							
Vehicle (MLA)	6	10.6 ± 0.9	6.1 ± 0.4	6.2 ± 0.4	6.0 ± 0.5	6.0 ± 0.4	6.2 ± 0.4
MLA	6	11.8 ± 0.8	9.5 ± 0.9[a]	10.4 ± 1.0[a]	9.7 ± 1.0[a]	9.9 ± 1.0[a]	10 ± 0.9[a]
L-NAME	6	9.8 ± 0.6	6.0 ± 0.4	6.2 ± 0.5	6.4 ± 0.5	6.3 ± 0.5	6.4 ± 0.5
L-NAME and MLA	6	9.3 ± 0.6	5.4 ± 0.4[b]	5.5 ± 0.4[b]	5.6 ± 0.5[b]	5.7 ± 0.5[b]	5.5 ± 0.4[b]
Vehicle (Cap) and MLA	6	9.8 ± 0.7	8.2 ± 0.5	8.3 ± 0.5	8.4 ± 0.4	8.1 ± 0.4	8.3 ± 0.5
Cap and MLA	6	8.5 ± 0.5	5.1 ± 0.4[c]	5.1 ± 0.5[c]	5.6 ± 0.4[c]	5.8 ± 0.2[c]	5.8 ± 0.2[c]
Heart rate (beats/min)							
Vehicle (MLA)	6	321 ± 13	255 ± 18	279 ± 16	269 ± 13	264 ± 10	260 ± 10
MLA	6	331 ± 12	318 ± 14	327 ± 19	307 ± 13	304 ± 13	305 ± 15
L-NAME	6	310 ± 16	235 ± 34	244 ± 30	251 ± 30	252 ± 31	247 ± 33
L-NAME and MLA	6	321 ± 16	253 ± 24	276 ± 17	280 ± 20	258 ± 24	283 ± 13
Vehicle (Cap) and MLA	6	330 ± 8	318 ± 13	327 ± 7	317 ± 4	329 ± 5	331 ± 14
Cap and MLA	6	315 ± 14	239 ± 16[c]	242 ± 15[c]	247 ± 15[c]	244 ± 16[c]	240 ± 19[c]

MLA: monophosphoryl lipid A (500 μg/kg); L-NAME: L-nitroarginine methyl ester (10 mg/kg); Cap: capsaicin (50 mg/kg).
[a] $P < 0.01$ vs. vehicle (MLA).
[b] $P < 0.01$ vs. MLA.
[c] $P < 0.01$ vs. Vehicle (Cap) and MLA.

kits were from Ju-Li Biological Medical Engineering Institute (Nanjing, P.R. China).

2.8. Statistics

All values are expressed as means ± S.E.M. 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

3.1. Myocardial function and release of creatine kinase

There were no significant differences in the basic values for 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 creatine kinase were shown during reperfusion after 4 h of ischemia. Pretreatment with monophosphoryl lipid A caused a significant improvement of the recovery of cardiac function and a decrease in the release of creatine kinase during reperfusion. The protective effects of monophosphoryl lipid A were abolished by pretreatment with L-NAME (Table 1 and Fig. 1).

In order to test the possible contribution of endogenous CGRP in the preconditioning with monophosphoryl lipid A, capsaicin, which selectively depletes the neurotransmitters in sensory nerves, was used. Pretreatment with capsaicin also prevented the protective effects of monophosphoryl lipid A. Capsaicin vehicle alone had no effect on the cardioprotection by monophosphoryl lipid A (Table 1 and Fig. 1).

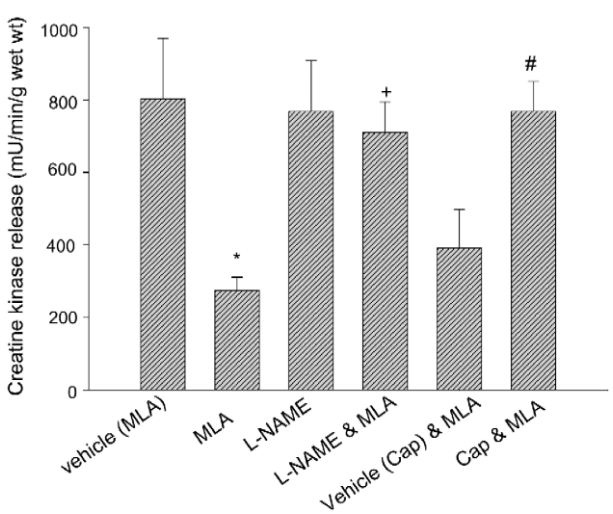

Fig. 1. Effect of monophosphoryl lipid A on creatine kinase release. Data were expressed as means ± S.E.M. ($n = 6$). MLA: monophosphoryl lipid A; L-NAME: L-nitroarginine methyl ester; Cap: capsaicin. $^{*}P < 0.01$ vs. vehicle (MLA), $^{+}P < 0.01$ vs. MLA, $^{\#}P < 0.01$ vs. vehicle (Cap) and MLA.

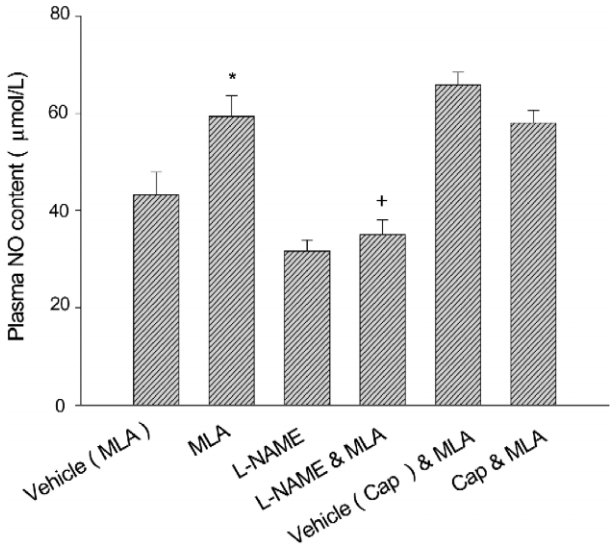

Fig. 2. Effect of monophosphoryl lipid A on plasma concentrations of NO. Data were expressed as means ± S.E.M. ($n = 6$). MLA: monophosphoryl lipid A; L-NAME: L-nitro arginine methyl ester; Cap: capsaicin. $^{*}P < 0.05$ vs. vehicle (MLA), $^{+}P < 0.01$ vs. MLA.

3.2. Plasma concentrations of NO and CGRP

Plasma concentrations of NO in the rats pretreated with monophosphoryl lipid A were increased significantly compared with those in the control group. The increase in the level of NO with monophosphoryl lipid A was abolished by pretreatment with L-NAME. However, pretreatment with capsaicin had no effect on the level of NO increased by monophosphoryl lipid A (Fig. 2).

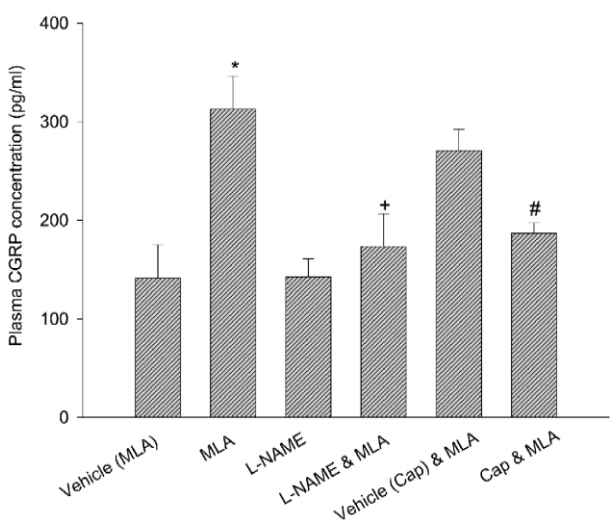

Fig. 3. Effect of monophosphoryl lipid A on plasma concentrations of CGRP. Data were expressed as means ± S.E.M. ($n = 6$). MLA: monophosphoryl lipid A; L-NAME: L-nitroarginine methyl ester; Cap: capsaicin. $^{*}P < 0.05$ vs. vehicle (MLA), $^{+}P < 0.01$ vs. MLA, $^{\#}P < 0.01$ vs. vehicle (Cap) and MLA.

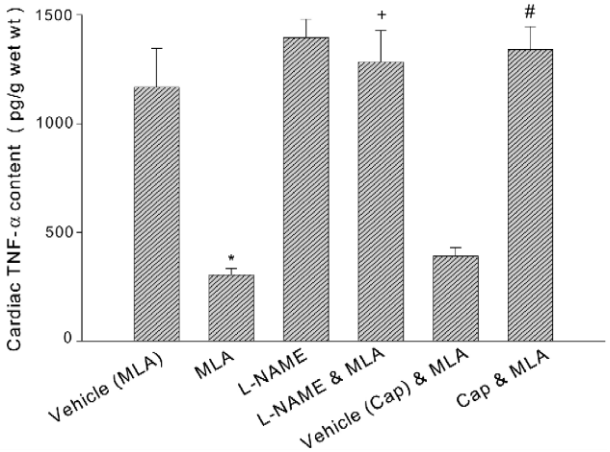

Fig. 4. Effect of monophosphoryl lipid A on the TNF-α content in cardiac tissues. Data were expressed as means ± S.E.M. ($n = 6$). MLA: monophosphoryl lipid A; L-NAME: L-nitroarginine methyl ester; Cap: capsaicin. $^{*}P < 0.01$ vs. vehicle (MLA), $^{+}P < 0.01$ vs. MLA, $^{\#}P < 0.01$ vs. vehicle (Cap) and MLA.

Treatment with monophosphoryl lipid A significantly increased the plasma concentrations of CGRP, an effect which was prevented by L-NAME or capsaicin (Fig. 3).

3.3. Myocardial TNF-α content

As shown in Fig. 4, reperfusion after 4 h of hypothermic ischemia caused a profound increase in the content of cardiac TNF-α. Pretreatment with monophosphoryl lipid A significantly attenuated the elevation of TNF-α content in the ischemic myocardium. However, the effect of monophosphoryl lipid A was abolished in the rats treated with L-NAME or capsaicin.

4. Discussion

Previous investigations have shown that ischemic (Lu et al., 1996) or hypoxic preconditioning (Engelman et al., 1995), as well as heat stress (Song et al., 1999b) improve the cardioprotection afforded by crystalloid cardioplegia, and that the protective effects of preconditioning have been thought to be mediated by endogenous chemical mediators. It has been reported that substitution of some drugs for ischemic stimulus can induce preconditioning-like protection and is also capable of enhancing protection with cardioplegia against myocardial injury due to hypothermic ischemia. In the present study, pretreatment with monophosphoryl lipid A, which induced a delayed preconditioning, significantly improved preservation with cardioplegia in the isolated rat heart, as shown by improvement of the recovery of cardiac function and reduction of creatine kinase release. These results suggest that pharmacological preconditioning, early or late, improves preservation with cardioplegia.

The preconditioning of the heart by monophosphoryl lipid A, a detoxified derivative of endotoxin has been demonstrated in the ischemia–reperfusion models in mice (Xi et al., 1999), rats (Maulik et al., 1995), rabbits (Zhao et al., 1996; Janin et al., 1998) and dogs (Przyklenk et al., 1997). There is substantial evidence to suggest that the beneficial effect of monophosphoryl lipid A is mediated by endogenous NO production, which is documented by previous observations that the effect of monophosphoryl lipid A is abolished in the presence of NO inhibitors (Toski, et al., 1998) or in the inducible nitric oxide synthase (iNOS) knockout mice (Xi et al., 1999).

NO, besides regulating vascular smooth muscle tone and protecting myocytes and endothelial cells, is also capable of modulating neurotransmission in central and peripheral nerves (Bredt et al., 1990; Huges and Brain, 1994). It has also been suggested that endogenous NO can modulate peptidergic neurotransmission in the rat stomach (Willis et al., 1996). Previous investigations have shown that endotoxin increases the release of CGRP (Wang et al., 1996), and that nitroglycerin, a NO donor, also significantly evokes the release of CGRP in central and peripheral vessels (Wei, et al., 1992; Fanciullacci et al., 1995; Booth et al., 2000). Our recent work has shown that, in the isolated rat heart, the protective effects of nitroglycerin-induced preconditioning are mediated by endogenous CGRP (Hu et al., 1999). In order to test whether the cardioprotection by monophosphoryl lipid A-induced delayed preconditioning is secondary to the release of CGRP, we used capsaicin (Källner and Franco-Cereceda, 1998). The results revealed that pretreatment with monophosphoryl lipid A caused an increase in the contents of NO and CGRP concomitantly with an improvement of the recovery of cardiac function, and pretreatment with capsaicin to deplete transmitters in sensory nerves abolished the protection and the increased release of CGRP induced by monophosphoryl lipid A, but did not affect the content of NO. In the case of L-NAME, the stimulated release of NO and CGRP and the protection afforded by monophosphoryl lipid A were abolished in the presence of L-NAME, an inhibitor of NO. These results support the hypothesis that monophosphoryl lipid A increases the release and synthesis of NO, with a subsequent stimulation of CGRP release, resulting in cardioprotection.

TNF-α, as an autocrine cytokine, has been shown to be released from the ischemic heart and to be involved in myocardial dysfunction in ischemia and reperfusion (Gurevitch et al., 1997). Previous studies have shown that an increased level of TNF-α is localized in cardiomyocytes from human myocardial tissues following acute ischemia, and pretreatment with neutralising anti-TNF-α antibody reduces myocardial damage and improves left-ventricular function during ischemia (Bozkurt et al., 1998). Recently, it has been found that the cardioprotection by ischemic preconditioning is related to the reduction of TNF-α production in the ischemic myocardium, and TNF-

α has been described as an ultimate effector in signal transduction pathways of ischemic preconditioning (Meldrum et al., 1998). More recently, it has been suggested that CGRP-mediated ischemic preconditioning is related to the inhibition of myocardial TNF-α production (Peng et al., 2000). As has been reported previously (Gurevitch et al., 1997), in the present study ischemia–reperfusion caused a significant increase in the content of TNF-α in the ischemic myocardium. Pretreatment with monophosphoryl lipid A markedly attenuated the increase in TNF-α content, which was abolished by capsaicin or L-NAME. These results suggest that the CGRP-mediated protection by monophosphoryl lipid A-induced preconditioning may be associated with inhibition of TNF-α production.

In conclusion, the present results suggest that the delayed preconditioning of the heart by monophosphoryl lipid A significantly improves preservation with cardioplegia and that the protective effects are mediated by endogenous CGRP.

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