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Involvement of α-calcitonin gene-related peptide in monophosphoryl lipid A-induced delayed preconditioning in rat hearts

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Abstract

Recent study has shown that monophosphoryl lipid A-induced delayed preconditioning enhanced preservation with cardioplegia and that the protective effects of monophosphoryl lipid A were related to stimulation of calcitonin gene-related peptide (CGRP) release. The purpose of the present study was to explore whether the elevated release of CGRP induced by monophosphoryl lipid A is secondary to stimulation of CGRP synthesis via the nitric oxide (NO) pathway and to characterize the isoform of CGRP. Sprague–Dawley rats were pretreated with monophosphoryl lipid A 24 h before the experiment, and then the left main coronary artery of rat hearts was subjected to 1 h occlusion followed by 3 h reperfusion. Infarct size, plasma creatine kinase activity, the plasma level of CGRP, and the expression of CGRP isoforms (α- and β-CGRP) mRNA in lumbar dorsal root ganglia were measured. Pretreatment with monophosphoryl lipid A (500 μg/kg, i.p.) significantly reduced infarct size and creatine kinase release. Monophosphoryl lipid A caused a significant increase in the expression of α-CGRP mRNA, but not of β-CGRP mRNA, concomitantly with an increase in plasma concentrations of CGRP, and the increased level of CGRP expression happened before stimulation of CGRP release. The effect of monophosphoryl lipid A was completely abolished by pretreatment with L-nitroarginine methyl ester (L-NAME, 10 mg/kg, i.p.), an inhibitor of NO synthase or capsaicin (50 mg/kg, s.c.), which selectively depletes transmitters in capsaicin-sensitive sensory nerves. The results suggest that the delayed cardioprotection afforded by monophosphoryl lipid A involves the synthesis and release of CGRP via the NO pathway, and that the protection is mainly mediated by the α-CGRP isoform.

Keywords: CGRP (calcitonin gene-related peptide); Monophosphoryl lipid A; Preconditioning; Ischemia–reperfusion; Capsaicin; Nitric oxide (NO)

1. Introduction

Exposing the heart to brief periods of ischemia followed by reperfusion renders the myocardium tolerant to sustained ischemia. This phenomenon, termed ischemic preconditioning, has been demonstrated in various animal species and in humans (Yellon et al., 1998). It has been reported that ischemic stimulus not only triggers early preconditioning, but also induces delayed preconditioning or "second window of protection". It has been shown that substitution of some drugs for ischemic stimulus is also capable of inducing preconditioning-like protection, termed pharmacological preconditioning. The mechanisms responsible for the protective effects of early and delayed preconditioning are not yet fully understood. There is abundant evidence to suggest that endogenous active substances including neurotransmitters and autacoids are involved in the mediation of ischemic or pharmacological preconditioning (Parratt, 1994).

Monophosphoryl lipid A, a detoxified derivative of endotoxin, has been shown to induce delayed cardioprotection in rats, rabbits, and dogs (Elliott, 1997). The mechanism of monophosphoryl lipid A-induced delayed preconditioning is not well understood although several possibilities have been suggested. Recently, several lines of study have shown that monophosphoryl lipid A-induced delayed preconditioning was related to stimulation of nitric oxide (NO) production (Zhao et al., 1997; Tosaki et al., 1998).

Calcitonin gene-related peptide (CGRP), a major transmitter in capsaicin-sensitive sensory nerves, has been shown to participate in the mediation of ischemic preconditioning (Li et al., 1996; Ferdinandy et al., 1997; Lu et al., 1999). Previous studies have shown that NO was involved in endotoxin-induced CGRP release (Wang et al., 1996; Tang et al.,

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1997). The cardioprotection afforded by nitroglycerin, a NO donor, was also related to stimulation of CGRP release (Hu et al., 1999). More recently, our study has demonstrated that monophosphoryl lipid A-induced delayed preconditioning enhanced preservation with cardioplegia and that the protective effects of monophosphoryl lipid A were related to stimulation of CGRP release via the NO pathway (He et al., 2001).

The purpose of the present study was 3-fold. First, since the delayed preconditioning of the heart induced by ischemic stimulus or drugs is related to stimulation of synthesis of some protective proteins, we tested whether the elevated release of CGRP induced by monophosphoryl lipid A is secondary to stimulation of CGRP synthesis. Secondly, since the α- and β-CGRP isoforms are encoded in different genes and there are some differences in physiological properties between α- and β-CGRP, we examined the effect of monophosphoryl lipid A on two isoforms (α and β) of CGRP mRNA expression. Thirdly, the effect of NO on the synthesis and release of CGRP was also tested.

2. Methods and materials

All animals received humane care in compliance with the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (NIH publication 86-23, revised 1986).

2.1. Experimental protocols

Male Sprague–Dawley rats weighing 280–320 g were randomly divided into eight groups. (1) The sham group underwent surgical procedures but without ischemic insult. (2) The ischemia–reperfusion group was subjected to 1 h of ischemia followed by 3 h of reperfusion. (3) The monophosphoryl lipid A group was pretreated with monophosphoryl lipid A (500 μg/kg, i.p.) 24 h before ischemia–reperfusion (He et al., 2001). (4) The monophosphoryl lipid A vehicle group was pretreated with a vehicle (containing 40% propylene glycol and 10% ethanol) 24 h before ischemia–reperfusion. (5) The monophosphoryl lipid A plus L-nitroarginine methyl ester (L-NAME) group was pretreated with L-NAME (10 mg/kg, i.p.) and monophosphoryl lipid A 24 h before ischemia–reperfusion (He et al., 2001). (5) The L-NAME group was pretreated with L-NAME 24 h before ischemia–reperfusion. (7) The capsaicin plus monophosphoryl lipid A group was pretreated with capsaicin (50 mg/kg, dissolved in a vehicle containing 10% Tween 80, 10% ethanol, and 80% saline, s.c.) 4 days before the experiment (Kallner and Franco-Cereceda, 1998), and then treated with monophosphoryl lipid A 24 h before coronary artery occlusion. (8) The capsaicin vehicle plus monophosphoryl lipid A group was treated with vehicle and monophosphoryl lipid A according to the same procedure as that in the capsaicin plus monophosphoryl lipid A group. All rats were subjected to coronary artery occlusion for 1 h and reperfusion for 3 h, except the sham group.

The second series of experiments was designed to evaluate the effect of monophosphoryl lipid A on the release and synthesis of CGRP. The same protocol was used as in the first series of experiments apart from the surgical procedure.

2.2. Surgical preparation

Rats were anesthetized with sodium pentobarbital (60 mg/kg, i.p.), and then mechanically ventilated with room air using a positive pressure ventilator. The ventilation rate was maintained at 30–35 strokes min^{-1} with a tidal volume of approximately 15 ml/kg body weight. Electrocardiograph leads were connected to the chest and limbs for continuous electrocardiograph monitoring throughout the experiment. A left thoracotomy was performed in the fourth intercostal space and the pericardium was opened to expose the heart. A 4-0 silk suture was passed around the left coronary artery and a snare was formed by passing both ends of the suture through a piece of polyethylene tubing. Occlusion of the coronary artery, by clamping the snare against the surface of the heart, resulted in an area of epicardial cyanosis with regional hypokinesis and electrocardiograph changes. Reperfusion was achieved by releasing the snare and was confirmed by conspicuous hyperaemic blushing of the previously ischemic myocardium and gradual resolution of the changes in the electrocardiograph signal. The sham group underwent the same procedure but without clipping of the coronary artery.

2.3. Infarct size and risk area

At the end of 3 h reperfusion, blood samples were collected from the carotid artery. The left coronary was reoccluded, and 1 ml Evans blue (1%) was injected into the left ventricular cavity in vivo and allowed to perfuse the non-ischemic portions of the heart. Then the rats were killed and the entire heart was weighed, excised, rinsed of excess blue dye, trimmed of right ventricular and atrial tissue, and sliced into 1-mm-thick sections from the apex to base. The slices were incubated in 1% triphenyl tetrazolium chloride solution at 37 °C for 20 min to stain the viable myocardium brick red. The samples were then fixed in a 10% formalin solution for 24 h. The sections were traced onto acetate sheets, then scanned into a computer and measured with an imaging analysis software (HPIAS-1000 imaging analysis system, Tongji Huahai, Wuhan, P.R. China). Infarct and risk area volumes were expressed as cubic centimeters and the percentage of infarct to risk area (ischemia–reperfusion) was calculated.

2.4. Creatine kinase assay

At the end of 3 h reperfusion, serum creatine kinase activity was measured spectrophotometrically.

2.5. Measurement of plasma NO concentrations

At the end of 3 h reperfusion, the serum level of NO, based on the content of nitrite and nitrate was measured with the nitratase assay.

2.6. RNA preparation and reverse transcription-polymerase chain reaction (RT-PCR)

After pretreatment with monophosphoryl lipid A for 0, 8, 12, 16 or 24 h, blood samples (3 ml) were withdrawn from the carotid artery for measurement of CGRP concentration under anesthesia, and then lumbar dorsal root ganglia were rapidly removed and homogenized in Trizol reagent. Total RNA isolation and semiquantitative RT-PCR were performed according to standard techniques.

The specific primer pairs and the size of the expected products were as follows (forward and reverse, respectively): α-CGRP, 5′-AAGTTCTCCCCTTTCCTGGT-3′ and 5′-GGTGGGCACAAAGTTGTCCT-3′ (318 bp); β-CGRP, 5′-GAGACCTTCAACACCCCAGCC-3′ and 5′-GGTGGGCACAAAGTTGTCCT-3′ (264 bp); β-actin, 5′-GAGACCTTCAACACCCCAGCC-3′ and 5′-TCGGGGCATCGGAACCGCTCA-3′ (422 bp) (Amara et al., 1985).

The PCR amplification profiles consisted of denaturation at 94 °C for 30 s, annealing at 58 °C for 30 s, and elongation at 72 °C for 45 s. The linear exponential phases for CGRP (α and β) and β-actin PCR were 28 and 25 cycles, respectively. Equal amounts of corresponding α-CGRP, β-CGRP or β-actin RT-PCR products were loaded on 1.7% agarose gels. Optical densities of ethidium bromide-stained DNA bands were quantitated and the results were expressed as CGRP/β-actin ratios.

2.7. Measurement of plasma CGRP level

The blood samples (3 ml) were placed in tubes containing 10% Na_2EDTA 40 μl and aprotinin 400 mU/l. Plasma was obtained by centrifugation at $1300 \times g$ for 20 min (4 °C). The plasma concentration of CGRP-like immunoreactivity was determined with radioimmunoassay kits, using antisera raised against rat CGRP, ^{125}I-labelled CGRP and rat CGRP standard.

2.8. Reagents

Capsaicin, monophosphoryl lipid A, L-NAME, triphenyl tetrazolium chloride and Evans blue were purchased from Sigma (St Louis, MO, USA). Creatine kinase assay kits were obtained from Zhongsheng Bioengineering (Beijing, P.R. China). NO assay kits were provided by Ju-Li Biological Medical Engineering Institute (Nanjing, P.R. China). Radioimmunoassay kits for measurement of CGRP were purchased from the Immunity Institute of Dongya (Beijing, P.R. China). Primers for PCR were synthesized in Sangon (Shanghai, P.R. China). Trizol reagent was obtained from GIBCOBRL (USA). The RT-PCR kits were purchased from Division of TaRaKa (Daliang, P.R. China).

2.9. Statistics

The data are expressed as means ± S.E.M. All values were analyzed using an analysis of variance (ANOVA) and the Student Newman–Keuls *t*-test. $P < 0.05$ was regarded as significant.

3. Results

3.1. Infarct size

As shown in Table 1, there were no differences in heart wet weights and risk zone among groups ($P > 0.05$), indicating that the size of the risk zone was comparable in all groups. Ischemia–reperfusion caused 67.3 ± 6.3% necrosis in the area at risk. Pretreatment with monophosphoryl lipid A caused a dramatic reduction in infarct size (31.5 ± 5.8%), an effect that was abolished by L-NAME (69.4 ± 6.6%). The vehicle of monophosphoryl lipid A or L-NAME alone had no effect on infarct size (65.0 ± 3.4% or 69.2 ± 6.2%). Pretreatment with capsaicin, which depletes transmitters in sensory nerves, also completely abolished the decrease in infarct size by monophosphoryl lipid A (62.3 ± 6.6%). The vehicle of capsaicin had no effect on protection by monophosphoryl lipid A (Fig. 1).

3.2. Creatine kinase release

Ischemia–reperfusion caused a significant increase in the serum level of creatine kinase. Pretreatment with monophosphoryl lipid A dramatically reduced the release of creatine kinase, an effect which was also abolished by L-NAME or capsaicin pretreatment. The vehicle of mono-

Table 1
Heart wet weight, area at risk and infarct size of each group

Group	*n*	Heart wet weight (g)	Area at risk (cm^3)	Infarct size (cm^3)
Ischemia–reperfusion	6	1.12 ± 0.06	0.41 ± 0.04	0.28 ± 0.03
MLA	6	1.09 ± 0.07	0.43 ± 0.02	0.14 ± 0.02[a]
Vehicle (MLA)	6	1.18 ± 0.09	0.40 ± 0.03	0.26 ± 0.01
MLA + L-NAME	6	1.10 ± 0.08	0.40 ± 0.04	0.28 ± 0.02[b]
L-NAME	6	1.16 ± 0.05	0.41 ± 0.02	0.28 ± 0.01
Cap + MLA	6	1.08 ± 0.08	0.39 ± 0.04	0.24 ± 0.01[b]
Vehicle (Cap) + MLA	6	1.14 ± 0.07	0.39 ± 0.04	0.13 ± 0.02

Values are means ± S.E.M., $n = 6$. 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. ischemia–reperfusion.

[b] $P < 0.01$ vs. MLA.

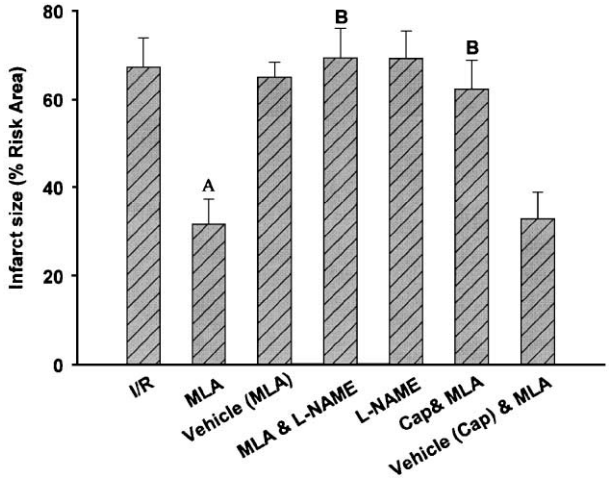

Fig. 1. Effect of monophosphoryl lipid A on myocardial infarct size, expressed as percentage of the area at risk. All values were expressed as means ± S.E.M. ($n=6$). I/R: ischemia–reperfusion; MLA: monophosphoryl lipid A; L-NAME: L-nitroarginine methyl ester; Cap: capsaicin. $^{A}P<0.01$ vs. I/R; $^{B}P<0.01$ vs. MLA.

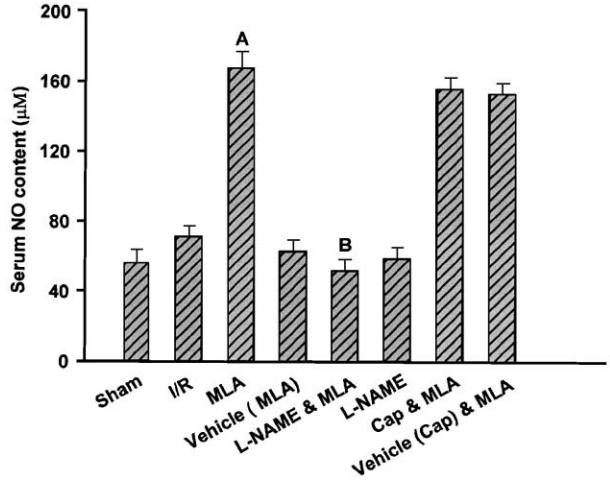

Fig. 3. Effects of monophosphoryl lipid A on serum concentrations of NO. All values were expressed as means ± S.E.M. ($n=6$). I/R: ischemia–reperfusion; MLA: monophosphoryl lipid A; L-NAME: L-nitroarginine methyl ester; Cap: capsaicin. $^{A}P<0.01$ vs. I/R; $^{B}P<0.01$ vs. MLA.

phosphoryl lipid A or L-NAME alone had no effect on the release of creatine kinase during reperfusion (Fig. 2).

3.3. Serum concentrations of NO

Serum concentrations of NO in the rats treated with monophosphoryl lipid A were significantly increased compared with those in the ischemia–reperfusion group. The increase in level of NO by monophosphoryl lipid A was abolished by treatment with L-NAME. However, pretreatment with capsaicin had no effect on the level of NO elevated by monophosphoryl lipid A (Fig. 3).

3.4. Plasma concentrations of CGRP

CGRP levels in plasma were significantly elevated after pretreatment with monophosphoryl lipid A for 12, 16 or 24 h, and the levels of plasma CGRP reached a peak at 16 h. However, the rise in the level of CGRP induced by monophosphoryl lipid A was blocked by pretreatment with L-NAME or capsaicin at mentioned time points (Fig. 4).

3.5. The level of CGRP mRNA

To test whether the elevated release of CGRP is due to stimulation of CGRP synthesis, the expression of CGRP

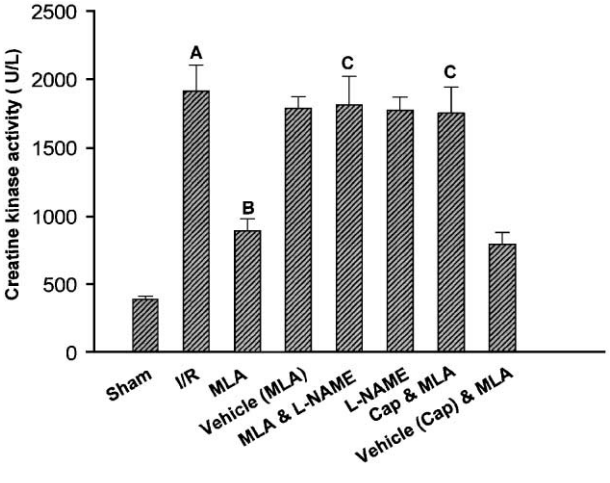

Fig. 2. Effect of monophosphoryl lipid A on creatine kinase activity. All values were expressed as means ± S.E.M. ($n=6$). I/R: ischemia–reperfusion; MLA: monophosphoryl lipid A; L-NAME: L-nitroarginine methyl ester; Cap: capsaicin. $^{A}P<0.01$ vs. sham; $^{B}P<0.01$ vs. I/R; $^{C}P<0.01$ vs. MLA.

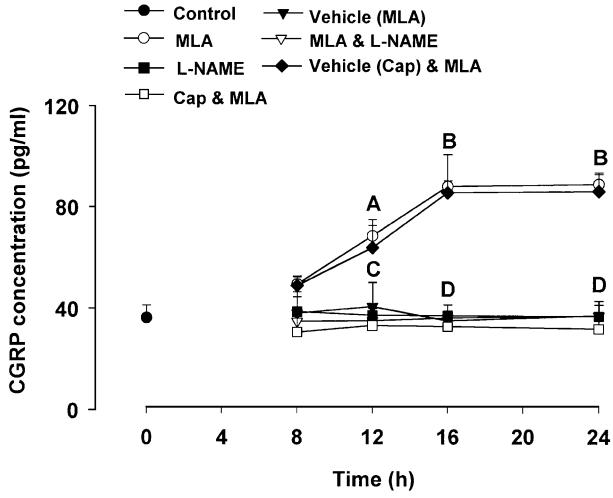

Fig. 4. Effects of monophosphoryl lipid A on plasma concentrations of CGRP. All values were expressed as means ± S.E.M. ($n=5$). MLA: monophosphoryl lipid A; L-NAME: L-nitroarginine methyl ester; Cap: capsaicin. $^{A}P<0.05$, $^{B}P<0.01$ vs. control; $^{C}P<0.05$, $^{D}P<0.01$ vs. MLA.

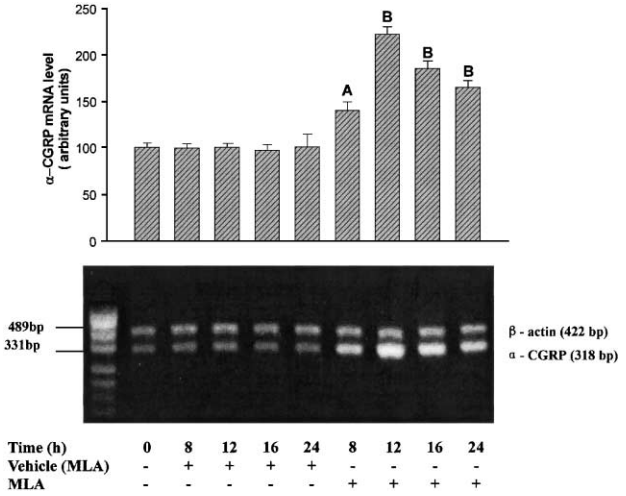

Fig. 5. Effect of monophosphoryl lipid A (MLA) on the level of α-CGRP mRNA in lumbar dorsal root ganglia. Rats were killed at the time indicated, and lumbar dorsal root ganglia were removed and total RNA isolated for RT-PCR analysis. The results of densitometric scanning (means ± S.E.M., $n=5$) for DNA bands of each group at each time point, expressed as α-CGRP/β-actin ratio, are shown in the bar graph above the print of RT-PCR products. $^{A}P<0.05$, $^{B}P<0.01$ vs. control.

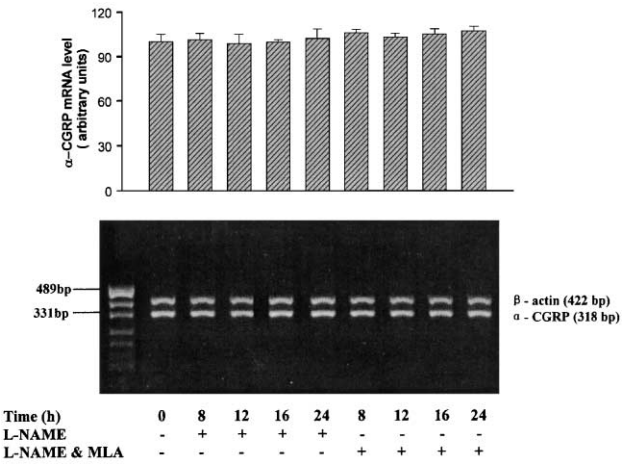

Fig. 7. Effect of L-nitroarginine methyl ester (L-NAME) on expression of α-CGRP mRNA in lumbar dorsal root ganglia in the presence or absence of monophosphoryl lipid A (MLA). Rats were killed at the time indicated, and lumbar dorsal root ganglia were removed and total RNA isolated for RT-PCR analysis. The results of densitometric scanning (means ± S.E.M., $n=5$) for DNA bands of each group at each time point, expressed as α-CGRP/β-actin ratio, are shown in the bar graph above the print of RT-PCR products.

mRNA in dorsal root ganglia was examined. Levels of α-CGRP mRNA were significantly increased by 40.0%, 122.5%, 85.6% or 65.0% after pretreatment with monophosphoryl lipid A for 8, 12, 16 or 24 h, respectively, as compared with control group (Fig. 5), whereas administration of monophosphoryl lipid A had no effect on the level of β-CGRP mRNA at any of the mentioned time points (Fig. 6). The level of α-CGRP mRNA reached a peak at 12 h after monophosphoryl lipid A administration, and still remained high at 24 h. The increase in level of α-CGRP mRNA by monophosphoryl lipid A was also completely blocked by pretreatment with L-NAME or capsaicin at any of the mentioned time points (Figs. 7 and 8).

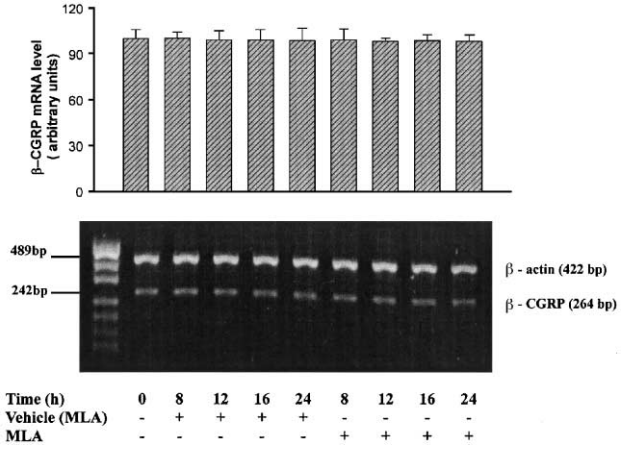

Fig. 6. Effect of monophosphoryl lipid A (MLA) on the level of β-CGRP mRNA in lumbar dorsal root ganglia. Rats were killed at the time indicated, and lumbar dorsal root ganglia were removed and total RNA isolated for RT-PCR analysis. The results of densitometric scanning (means ± S.E.M., $n=5$) for DNA bands of each group at each time point, expressed as β-CGRP/β-actin ratio, are shown in the bar graph above the print of RT-PCR products.

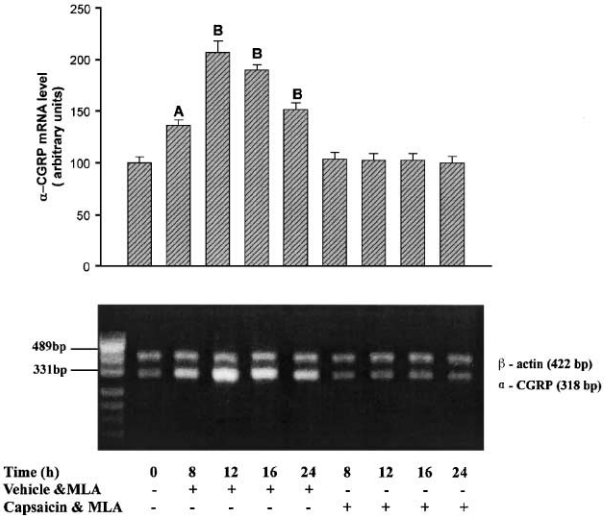

Fig. 8. Effect of capsaicin on expression of α-CGRP mRNA in lumbar dorsal root ganglia in the presence of monophosphoryl lipid A (MLA). Rats were killed at the time indicated, and lumbar dorsal root ganglia were removed and total RNA isolated for RT-PCR analysis. The results of densitometric scanning (means ± S.E.M., $n=5$) for DNA bands of each group at each time point, expressed as α-CGRP/β-actin ratio, are shown in the bar graph above the print of RT-PCR products. $^{A}P<0.05$, $^{B}P<0.01$ vs. control.

4. Discussion

In the present study, we have shown that both cardioprotection and stimulation of CGRP release induced by monophosphoryl lipid A were abolished by L-NAME, the NO synthase inhibitor, or capsaicin, which depletes transmitters in capsaicin-sensitive sensory nerves. Furthermore, we have characterized a CGRP isoform-mediated monophosphoryl lipid A-induced delayed preconditioning.

Monophosphoryl lipid A, a detoxified derivative of endotoxin, has been demonstrated to provide powerful protective effects in various animal species (Elliott, 1997). The results of the present study confirm previous observations that pretreatment with monophosphoryl lipid A significantly reduces infarct size and creatine kinase release due to ischemia–reperfusion, and the protection afforded by monophosphoryl lipid A was blocked by L-NAME. Others have demonstrated that the effect of monophosphoryl lipid A was abolished in inducible nitric oxide synthase (iNOS) knockout mice (Xi et al., 1999), in further support of the hypothesis that the delayed preconditioning of the heart with monophosphoryl lipid A is mediated by endogenous NO production.

CGRP, a 37-amino acid peptide, a principal transmitter in capsaicin-sensitive sensory nerves is widely distributed in cardiovascular tissues (Franco-Cereceda, 1988). CGRP has two isoforms named α- and β-CGRP, which are encoded in different genes and differ from each other by only one or three amino acids in rats or humans (Kallner, 1998). CGRP exerts prominent chronotropic and smooth muscle relaxing effects, and is the most potent endogenous vasodilator yet discovered. There is evidence that exogenous administration of CGRP protects the myocardium against ischemia–reperfusion injury (Li et al., 1996). It has also been found that pretreatment with capsaicin aggravates myocardial infarction in the porcine heart, and one postulates that the elevated level of CGRP during ischemia probably constitutes a compensatory response (Kallner and Franco-Cereceda, 1998). Recently, we and others have shown that endogenous CGRP may be an important mediator in the cardioprotection of ischemic preconditioning. This is documented by previous observations that the protection afforded by ischemic preconditioning is abolished by CGRP-(8–37), the selective CGRP receptor antagonist, or by CGRP antibody, or by capsaicin (Li et al., 1996; Ferdinandy et al., 1997; Lu et al., 1999). CGRP is not only involved in the protection of early preconditioning, but also participates in the mediation of delayed preconditioning (Song et al., 1999). Our recent work has shown that the cardioprotection afforded by nitroglycerin is mediated by endogenous CGRP (Hu et al., 1999). Others have reported that nitroglycerin also significantly evokes the release of CGRP in central and peripheral vessels (Bredt et al., 1990; Huges and Brain., 1994). Previous investigations have shown that NO was involved in endotoxin-induced CGRP release (Wang et al., 1996; Tang et al., 1997). Based on the regulatory effect of NO, endogenous or exogenous, on CGRP release, we postulate that endogenous CGRP release may be involved in the delayed protection afforded by monophosphoryl lipid A. Our recent work has shown that monophosphoryl lipid A-induced delayed preconditioning enhanced preservation with cardioplegia and that the protective effects of monophosphoryl lipid A were related to stimulation of CGRP release via the NO pathway (He et al., 2001). The present results revealed that in vivo pretreatment with monophosphoryl lipid A caused an increase in blood concentrations of both NO and CGRP, concomitantly with a reduction of infarct size and creatine kinase release. However, the elevated levels of NO and CGRP as well as the cardioprotection by monophosphoryl lipid A were abolished in the presence of L-NAME. Pretreatment with capsaicin also abolished the rise in CGRP levels, but not those of NO, and the protection induced by monophosphoryl lipid A. These findings, together with abovementioned findings, support the hypothesis that the delayed preconditioning of the heart with monophosphoryl lipid A is mediated by endogenous CGRP via stimulation of NO production.

Growing evidence has suggested that changes in the gene expression of some protective substances are necessary for the development of delayed preconditioning (Rizvi et al., 1999). It is probable that the elevated release of CGRP induced by monophosphoryl lipid A is secondary to stimulation of CGRP synthesis. Therefore, we tested the effect of monophosphoryl lipid A on the expression of CGRP mRNA. It is known that the α- and β-CGRP isoforms are encoded in different genes and differ from one another by only one or three amino acids in rats or humans. Both α- and β-CGRP are synthesized in the cell bodies of primary sensory neurons and transported axonally to mainly peripheral but also central nerves terminals, where it is stored in large, dense-cored secretory granules (Kallner, 1998). Thus, in the present study, changes in the expression of α- and β-CGRP mRNA in dorsal root ganglia, a major site of CGRP synthesis, were compared. The results showed that levels of α-CGRP mRNA in dorsal root ganglia were increased significantly in the rats treated with monophosphoryl lipid A, whereas no changes in the expression of β-CGRP mRNA were observed. The increased expression of α-CGRP mRNA happened before stimulation of CGRP release. The effect of monophosphoryl lipid A was completely blocked by pretreatment with L-NAME or capsaicin. These results suggest that the delayed preconditioning induced by monophosphoryl lipid A is related to the release and synthesis of CGRP. However, the precise mechanism responsible for NO-mediated α-CGRP mRNA expression needs to be investigated.

Of the two isoforms, α-CGRP seems to be predominant in sensory neurons, because analysis of RNA from various regions of the nervous system has shown that the level of β-CGRP mRNA is 20% lower than that of α-CGRP mRNA (Gibson et al., 1988). The present results are consistent with previous observations that the level of β-CGRP mRNA was apparently lower than that of α-CGRP mRNA in the rat. Previous studies suggested that there were no distinguishable differences in biological activity between α- and β-CGRP

(Mclatchine et al., 1998). However, recent investigations have shown that only α-CGRP elicits effects on axonal transport in sensory neurons (Hiruma et al., 2000), and only α-CGRP mRNA has been detected in rat hearts and the enteric nervous system of rat small intestine (Ramana et al., 1992; Doi et al., 2000), and only β-CGRP mRNA, but not α-CGRP mRNA, has been detected in rat T lymphocytes (Xing et al., 2000). These results indicate that there are some unknown differences in biological actions between α- and β-CGRP. In the present study, monophosphoryl lipid A induced only α-CGRP but not β-CGRP mRNA expression, suggesting that the cardioprotection afforded by monophosphoryl lipid A is mainly mediated by the α-CGRP isoform in the rat.

It is noteworthy that capsaicin-sensitive sensory nerves contain a number of peptides, including CGRP, substance P and neurokinin A (Franco-Cereceda, 1988). Besides CGRP, substance P and neurokinin A could also mediate the effect of capsaicin. Among these peptides, CGRP has been shown to exert a beneficial effect on the myocardium. We and others have shown that, in the isolated rat heart, pretreatment with capsaicin protected against myocardial injury induced by ischemia–reperfusion, an effect which is due to stimulation of CGRP release in cardiac sensory nerves (D'Alonzo et al., 1995; Li et al., 1996). Although there is evidence that substance P or neurokinin A released from peripheral nerve endings of afferent nerve fibers causes only vasodilation, contraction of smooth muscles in bronchi (Maggie, 1988; Pernow, 1983), we cannot exclude the possibility that either substance P or neurokinin A is beneficial to the myocardium, although direct protection by either has not yet been reported.

Another interesting finding of the study is that capsaicin, besides stimulation of CGRP release, blocked the mRNA expression of α-CGRP induced by monophosphoryl lipid A. Capsaicin, which selectively stimulates the release of sensory nerve transmitters, including CGRP, has been widely used as a tool to study sensory transmission. Previous investigations have shown that pretreatment with capsaicin (total dose 50 mg/kg, s.c.) 2 weeks before the experiment caused an almost total loss of substance P- and CGRP-immunoreactive nerves within the heart in the guinea pig or the rat (Franco-Cereda and Lundberg, 1988). Recently, others have shown that immunoreactive fibers in the atria were scarce in the rat treated with capsaicin 3 days before the experiment (Ferdinandy et al., 1997). Acute desensitization with capsaicin caused a reduction or loss of responses to various factors, such as electrostimulation (Li and Duckles, 1991), ischemia (Peng et al., 2000), hyperthermia (Song et al., 1999) or chemical substances (Hu et al., 1999), in the terminals of peripheral sensory nerves. More recently, it has been found that capsaicin receptors were also present in cell bodies of primary sensory nerves (Sasamura and Kuraishi, 1999). It is possible that capsaicin at high concentrations also causes desensitization in the cell bodies of sensory nerves, resulting in reduction or loss of responses to chemical mediators or drugs such as monophosphoryl lipid A. However, the precise mechanism responsible for inhibitory effect of capsaicin on CGRP expression still needs to be investigated.

The mechanisms responsible for the protective effects of CGRP also remain unclear. There is evidence suggesting that the K_{ATP} channel may be involved in monophosphoryl lipid A-induced cardioprotection in the rabbit and the dog (Elliott, 1997). However, numerous studies from different laboratories have demonstrated that the K_{ATP} channel does not participate in the cardioprotection by ischemic preconditioning in the rat (Grover et al., 1993; Lu et al., 1993). Recently, our work has shown that the cardioprotective effect afforded by CGRP-mediated ischemic preconditioning was related to inhibition of cardiac tumor necrosis factor-α production, an ultimate effector in signal transduction pathways of ischemic preconditioning (He et al., 2001; Peng et al., 2000; Meldrum et al., 1998).

In summary, the present results suggest that the delayed cardioprotection by monophosphoryl lipid A involves the synthesis and release of CGRP, and that the protection is mainly mediated by α-CGRP.

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