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Attenuation of post-ischemia reperfusion injury by thaliporphine and morphine in rat hearts

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Summary

Pretreatment with thaliporphine before ischemia affords cardioprotective effects against reperfusion injury via antioxidant activity. This study evaluated whether thaliporphine administered at a certain period after myocardial ischemia conferred the same cardioprotection and assessed its possible new mechanism. The left main coronary artery of anaesthetized rats was occluded for 1 h and then reperfused for 2 h. Thaliporphine was administered at 10 min before reperfusion. Controls received saline only. Morphine, a nonselective opioid receptor agonist, was used as reference compound at 0.3 mg/kg. Thaliporphine at 0.05 and 0.5 mg/kg were found to reduce the infarct size. Recovery of cardiac function was higher in thaliporphine (0.5 mg/kg) group, as assessed by a significant improvement in the rates of pressure development ($+dp/dt_{max}$). This compound also reduced plasma creatine kinase and cardiac MPO activity. These protective effects afforded by thaliporphine were diminished by the opioid receptor antagonists (naloxone or naltrexone) and by the mitochondrial K_{ATP} blocker 5HD. In comparison, morphine reduced infarct size and MPO activity in the myocardium but produced slightly improvement in cardiac function after ischemia-reperfusion. These results demonstrate that reperfusion therapy with thaliporphine protect cardiac injury through further mechanism via activation of opioid receptor and opening of mitochondrial K_{ATP} channels as morphine but with stronger activity.

Abbreviations: LVDP – left ventricular developed pressure; LVSP – left ventricular systolic pressure; $+dp/dt_{max}$ and $-dp/dt_{max}$ – the maximum and minimum first derivative of LVSP; CK – creatine kinase; 5-HD – 5-hydroxydecanoate; K_{ATP} channel – ATP sensitive potassium channel; MPO – Myeloperoxidase

Introduction

The timely restoration of flow following an acute coronary obstruction is a prerequisite for ischemic myocardial salvage. However, evidence from animal studies as well as clinical observations demonstrates that reperfusion itself may cause additional cardiac damage, defined as "reperfusion injury". The manifestations of reperfusion injury include arrhythmia, myocardial stunning, endothelial dysfunction and cell death [1, 2]. Therapeutic strategies that target mechanisms related to reperfusion-induced injury may provide new agents, which could be used as adjuncts to current reperfusion therapy, to limit myocardial damage [3].

Traditionally, opioids are used in the relief of pain. Opioids also protect the organs from hypoxia or ischemic insults [4–9]. A growing body of evidence supports the contention that opioid receptor stimulation may provoke ischemic preconditioning to protect against myocardial ischemia [10–12]. How-

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ever, clinically relevant studies examining opioid-induced preconditioning are limited. In acute myocardial infarction morphine is administered intravenously during or after, but rarely before myocardial ischemia [13]. Recently, it has been found that opioids can protect against post-ischemic myocardial infarction when given before reperfusion [14].

Thaliporphine is a phenolic aporphine alkaloid found in many medicinal plants such as *Lauracea* [15]. This compound has partial calcium (Ca^{2+}) channel-activating activity and strong sodium (Na^{+}) and potassium (K^{+}) channel-blocking activities, which may contribute to its antiarrhythmic action [16]. However, the Ca^{2+} channel-activating activity may increase the Ca^{2+} overload and induced trigger activity during myocardial ischemia-reperfusion. In contrast, a previous study found that pretreatment with thaliporphine can protect the myocardium from ischemic injury in a nitric oxide (NO)-dependent manner and prevent reperfusion injury via its antioxidant activity [17].

The objective of the present study was to further evaluate whether thaliporphine administered before coronary reperfusion could have the same cardioprotective effect as pretreatment before the ischemia-reperfusion period. Since thaliporphine is an aporphine alkaloid with a structure similar to morphine, and a binding affinity to opioid receptor by using radioligand binding assays was observed (unpublished observation), we examined whether it exerted cardiac protective activity through activation of the opioid receptor and opening of mitochondrial K_{ATP} channels, in a manner analogous to morphine [18, 19].

Methods

Animals

Male Sprague-Dawley rats (National Laboratory Animal Center, Taipei, Taiwan) weighing 250–350 g were used. The animals were housed in a conditioned environment (22 ± 1 °C, 55 ± 5% relative humidity, 12-h light and 12-h darkness cycle) and were given free access to food and tap water. The investigation conforms with the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH Publication No. 85–23, revised 1996). Throughout the studies, all efforts were taken to minimize animal pain and suffering.

General surgical preparation

Rats underwent myocardial ischemia by a temporary occlusion of the left main coronary artery as previously described [20]. Briefly, rats were intraperitoneally anesthetized with urethane (1.25 g/kg) and placed on an operating table. After tracheotomy, the animals were ventilated with room air by a rodent ventilator (Model 683, Harvard Apparatus, South Natick, MA) with a stroke volume of 10–12 ml/kg body weight at a rate of 65 strokes/min. The chest was opened and the ribs were gently spread. The heart was quickly expressed out of the thoracic cavity, inverted and a 7/0 silk ligature was placed under the left main coronary artery. The heart was repositioned in the chest and the animal was allowed to recover for 15 min. A small plastic snare formed from a Portex P-270 cannula was threaded through the ligature and placed in contact with the heart. The ligature was tightened to occlude the artery and reperfusion was initiated by withdrawing the polyethylene tubing. Regional myocardial ischemia was verified by the presence of a zone of cyanosis in the area of distribution of the occluded vessel and by changes in the electrocardiogram consistent with the presence of transmural regional myocardial ischemia (ST – segment elevation).

In some groups, a Millar catheter with a high fidelity pressure sensor (Model SPC 320, size 2F, Millar Instruments, Houston, TX) was inserted via the right carotid artery into the left ventricle. Before and after the ischemia-reperfusion, heart rate (HR), left ventricular developed pressure (LVDP), the maximum and minimum first derivative of LVSP ($+dp/dt_{max}$ and $-dp/dt_{max}$) and ECG changes were recorded on a personal computer with data analysis software (PowerLab data acquisition system, AD Instruments, Castle Hill, NSW, Australia).

Experimental Groups

Animals were randomly assigned to one of eleven groups (Figure 1), each of which received 1 h of coronary artery occlusion followed by 2 h of reperfusion. A bolus of morphine (0.3 mg/kg), thaliporphine (0.05; 0.5 mg/kg) or vehicle (saline, 0.9% NaCl) was infused from a jugular vein at 10 min before coronary reperfusion. To demonstrate the mechanism of the cardioprotective effect induced by thaliporphine, the following antagonists

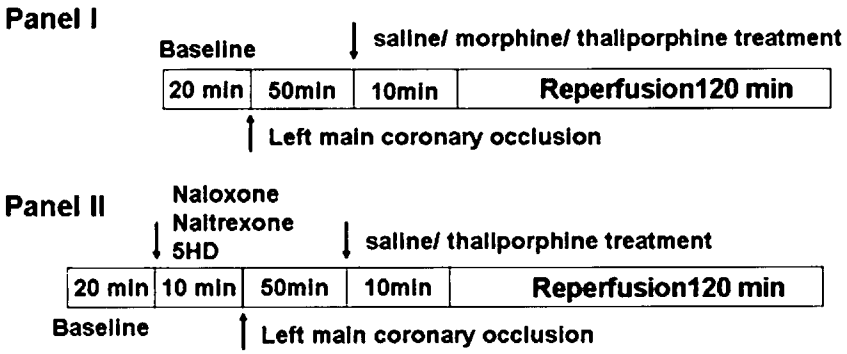

Figure 1. Experimental groups. All animals proceeded to coronary artery occluded for 1 h followed by 2 h of reperfusion were randomly assigned to one of eleven groups Panel I: Animals were infused with a bolus of morphine, thaliporphine (0.05; 0.5 mg/kg) or saline from a jugular vein 10 min before coronary reperfusion. To demonstrate the mechanism of the cardioprotective effect induced by thaliporphine, the following antagonists were used. The opioid antagonists, naloxone (1 mg/kg or 3 mg/kg) or naltrexone (2 mg/kg), and the mitochondrial K_{ATP} blocker (5HD; 5 mg/kg) was given 10 min before LAD occlusion (Panel II).

were used. The opioid antagonists, naloxone (1 or 3 mg/kg) and naltrexone (2 mg/kg), or a mitochondrial K_{ATP} blocker (5HD, 5 mg/kg) were administered at 10 min before LAD occlusion to ensure acting on the target of the ischemic-reperfusion zone. Doses of the antagonists used were those known to block cardiac preconditioning and had no effect on cardiac infarction under ischemia-reperfusion condition when administrated alone [10, 21].

Definition of area at risk and area of infarction

Infarct size and ischemic risk area were determined by the triphenyl tetrazolium staining technique [22]. At the end of reperfusion, the left coronary artery was re-occluded. Methyl blue dye (2 ml; 3% in 0.9% NaCl) was slowly infused from a jugular vein to delineate the risk area as a perfusion defect. The risk area was cut out, weighed, and expressed as a percentage of total ventricular weight. Thereafter, ventricular tissue was sliced into 1 mm sections for incubation in tetrazolium dye (10 mg 2,3,5- triphenyltetrazolium chloride (TTC)/ml 0.9% NaCl, pH 7.4) at 37 °C for 40 min. Sections were then placed in 10% formaldehyde for 2 days before the infarct (white) tissue was excised. The weight of infarct tissue was expressed as a percentage of occluded zone.

Plasma CK analysis

Cellular damage was evaluated by measuring the plasma CK. Blood samples were drawn from the carotid artery at the end of reperfusion and collected in heparinized tubes. The blood was then centrifuged at 1000*g* for 5 min. The plasma was kept at 4 °C until it was used for determination of CK activity with a commercial kit from Randox (Randox Laboratories Ltd., Crumlin, UK).

Myeloperoxidase activity

In control, morphine, 0.5 mg/kg thaliporphine, naloxone, naltrexone, 5HD, 0.5 mg/kg thaliporphine pretreated with naloxone, naltrexone or 5HD groups, cardiac tissue collected at 2 h after reperfusion was used for determination of MPO activity with commercial kit from Gene Research Lab (Taipei, Taiwan). Briefly, tissue samples were homogenized in hexadecyltrimethyl ammonium bromide and dissolved in potassium phosphate. After centrifugation, supernatants were collected and mixed with *o*-dianiside hydrochloride and hydrogen peroxide in phosphate buffer. The activity of MPO was measured spectrophotometrically at 405 nm absorbance. MPO activity was defined as the quantity of enzyme degrading 1 mmol of peroxide per min at 37 °C and was expressed in units per gram weight of wet tissue.

Chemicals

Thaliporphine was prepared from isoboldine, isolated from plant *Neolitsea konishii K*, by selective 9-O-methylation with diazomethane [23]. Urethane, morphine, naloxone, naltrexone, 5-hydroxydecanoate (5-HD), 2,3,5-triphenyl- tetrazolium chloride (TTC), were purchased from Sigma Chemical Co.

(St. Louis, MO) USA). All substances were dissolved in saline except for urethane, which was dissolved in distilled water.

Statistics

All values are presented as mean ± standard error. Differences between groups were assessed by one-way ANOVA and Student–Newman–Keuls multiple comparison test where appropriate. p values < 0.05 were considered as significant.

Results

Ischemia-induced arrhythmias

Ligation of the left coronary artery invariably resulted in ventricular arrhythmias, which commenced within 5–7 min of coronary occlusion. To exclude the influence of ischemia-induced arrhythmias on the following reperfusion therapy, we monitored the arrhythmias from EKG during coronary artery ligation period. The arrhythmia manifested as ventricular premature contraction, ventricular tachycardia and ventricular fibrillation (Table 1). Rats with parallel ischemic arrhythmia pattern were randomly assigned to one of the other ten groups. However, one exception displayed a prominent inhibition of ventricular fibrillation in 1 mg/kg naloxone pretreatment group. It has been reported that naloxone has antiarrhythmic activity via the inhibition of ionic channels [24].

Cardiac function recovery in ischemia-reperfusion

The data concerning HR, LVDP, $+dp/dt_{max}$ and $-dp/dt_{max}$ at baseline and after 120 min of reperfusion is summarized in Table 2.

There were no significant differences between groups at baseline. After ischemia-reperfusion, all parameters of cardiac function were markedly decreased in control group. The recovery of developed pressure and the rate of pressure relaxation ($-dp/dt_{max}$) were higher, but only the rate of pressure development ($+dp/dt_{max}$) was significantly improved in hearts of thaliporphine (0.5 mg/kg) group ($p < 0.05$). The recovery of cardiac function, especially the rate of pressure development ($+dp/dt_{max}$), stimulated by thaliporphine in ischemia-reperfusion was attenuated by the opioid receptor antagonists naloxone ($p < 0.05$) or naltrexone ($p < 0.05$) and the mitochondrial K_{ATP} blocker 5HD ($p < 0.05$). All antagonists administrated alone had no effect on the cardiac dysfunction of ischemia-reperfusion when compared with control group. In addition, morphine administration 10 min before reperfusion slightly improved cardiac function recovery after ischemia-reperfusion when compared with control group.

Myocardial infarct size

The area at risk (Figure 2a) was similar among groups. The infarct size was 44.2 ± 2.5% in the control group (Figure 2b). Thaliporphine administered at 0.05 and 0.5 mg/kg reduced myocardial

Table 1. Incidence and duration of ventricular arrhythmia induced by left coronary artery occlusion in difference groups.

Group	Ventricular tachycardia		Ventricular fibrillation		VPC (beats)
	Incidence (%)	Duration (s)	Incidence (%)	Duration (s)	
Control	100	151.72 ± 23.53	63	97.39 ± 20.79	230 ± 59
Morphine	100	135.62 ± 20.18	100	98.13 ± 13.92	224 ± 23
Thali 0.05 mg/kg	100	79.62 ± 30.32	75	32.27 ± 16.93	170 ± 73
Thali 0.5 mg/kg (1)	100	112.06 ± 41.13	50	80.72 ± 20.62	218 ± 66
(1) + Nlx 1 mg/kg	100	108.23 ± 44.91	–	–	143 ± 30
(1) + Nlx 3 mg/kg	100	112.19 ± 18.65	86	93.48 ± 6.70	261 ± 39
(1) + Ntx 2 mg/kg	100	83.67 ± 37.20	55	74.62 ± 25.38	214 ± 51
(1) + 5HD	100	158.77 ± 27.92	75	74.94 ± 28.23	382 ± 84
Nlx 3 mg/kg	100	105.42 ± 37.25	80	68.19 ± 29.82	157 ± 25
Ntx 2 mg/kg	100	152.90 ± 28.20	64	89.95 ± 10.04	245 ± 59
5HD 5 mg/kg	100	125.05 ± 39.96	100	55.47 ± 11.48	202 ± 36

Data are presented as mean ± s.e.

Table 2. Effects of morphine, thaliporphine and thaliporphine plus naloxone, naltrexone or 5HD on myocardial contractile function in anesthetized rats subjected to ischemia-reperfusion.

Group	Heart rate (bpm)	Pre-I/R			Heart rate (bpm)	Post-I/R		
		LVDP (mmHg)	$+dp/dt_{max}$ (mmHg/s)$\times 10^3$	$-dp/dt_{max}$ (mmHg/s)$\times 10^3$		LVDP (mmHg)	$+dp/dt_{max}$ (mmHg/s)$\times 10^3$	$-dp/dt_{max}$ (mmHg/s)$\times 10^3$
Control	384 ± 14	106 ± 3	9.7 ± 0.9	5.5 ± 0.5	298 ± 16*	78 ± 6*	4.1 ± 0.2*	3.1 ± 0.3*
Morphine 0.3 mg/kg	383 ± 8	99 ± 2	8.0 ± 0.5	4.5 ± 0.4	311 ± 13*	82 ± 8	4.6 ± 0.5*	3.6 ± 0.5
Thali 0.05 mg/kg	410 ± 16	111 ± 3	10.2 ± 0.6	5.8 ± 0.6	327 ± 27*	86 ± 1*	4.8 ± 0.2*	3.3 ± 0.3*
Thali 0.5 mg/kg (1)	411 ± 6	106 ± 8	10.1 ± 0.1	5.0 ± 0.7	340 ± 6	90 ± 5	5.7 ± 0.5*†	4.6 ± 0.4
(1) + Nlx	367 ± 5	100 ± 2	8.0 ± 0.4	4.4 ± 0.1	342 ± 5	79 ± 5	4.0 ± 0.3*§	3.5 ± 0.3
(1) + Ntx	393 ± 19	105 ± 3	10.7 ± 0.6	5.3 ± 0.2	353 ± 22*	71 ± 0.3*	4.2 ± 0.3*§	3.4 ± 0.1*
(1) + 5HD	379 ± 13	101 ± 3	7.8 ± 0.7	4.9 ± 0.2	326 ± 29	71 ± 9*	3.6 ± 0.5*§	3.0 ± 0.7
Nlx3 mg/kg	400 ± 10	105 ± 5	9.8 ± 0.6	5.7 ± 0.3	353 ± 11	79 ± 2*	4.1 ± 0.3*§	3.6 ± 0.4*
Ntx 2 mg/kg	407 ± 13	108 ± 3	10.9 ± 3	5.8 ± 0.3	396 ± 22	78 ± 2*	4.4 ± 0.1*§	3.5 ± 0.2*
5HD 5 mg/kg	401 ± 21	102 ± 5	10.3 ± 0.7	5.5 ± 0.6	328 ± 19*	77 ± 5*	4.1 ± 0.2*§	3.0 ± 0.4*

Data are presented as mean ± s.e.
*Significant difference from the baseline (pre-*I/R*) values.
†Significant difference from control group.
§Significant difference from 0.5 mg/kg thaliprophine group.

infarction (31.6 ± 3.3% and 22.6 ± 2.2%, $p < 0.01$ vs. control group, respectively).

The anti-infarction of thaliporphine was diminished by naloxone. The low dose of naloxone (1 mg/kg) partially antagonized the anti-infarct action of thaliporphine (29.9 ± 1.6%, $p < 0.05$ vs. 0.5 mg/kg thaliporphine group). The high dose of naloxone (3 mg/kg) abrogated more but not completely of the anti-infarct action of thaliporphine (37.9 ± 1.5%, $p < 0.01$ vs 0.5 mg/kg thaliporphine group), since the infarct size of this group was significant different from the naloxone alone group ($p < 0.05$). Likewise, another opioid antagonist, naltrexone (2 mg/kg) or the mitochondrial K_{ATP} blocker, 5HD (5 mg/kg) effectively but not fully attenuated the infarct size reducing activity of thaliporphine (thaliporphine plus naltrexone group: 39.1 ± 1.5%, $p < 0.05$ vs. 0.5 mg/kg thaliporphine group; $p < 0.05$ vs. naltrexone alone group; thaliporphine plus 5HD group: 35.3 ± 1.6%, $p < 0.01$ vs. 0.5 mg/kg thaliporphine group; $p < 0.05$ vs. 5HD alone group).

For comparison, administration of morphine (0.3 mg/kg), an opioid receptor agonist, also reduced the infarct size to 31.2 ± 2.2% ($p < 0.01$ vs. control group) after ischemia-reperfusion.

Plasma CK activity

Plasma CK activity was used to confirm infarct size as quantified by TTC staining. A marked increase of this enzyme was found in the plasma of ischemia-reperfusion rats in the control group. (10424.3 ± 1180.3 U/l). Thaliporphine 0.05 and 0.5 mg/kg reduced plasma CK levels of ischemia-reperfusion rats to 8017.5 ± 1421.1 U/l and 3905.6 ± 508.4 U/l ($p < 0.01$ vs. control group). Treatment with 0.3 mg/kg morphine also showed a similar reduction effect on plasma CK levels (6641.0 ± 698.0 U/l), but which were not significant different from the control group ($p > 0.05$) (Figure 3).

In addition, the reduction of CK release in 0.5 mg/kg thaliporphine group was nearly completely abolished by 5HD (10162.9 ± 1711.3 U/l, $p < 0.01$ vs. 0.5 mg/kg thaliporphine group). The antagonistic effects of naloxone or naltrexone against thaliporphine inhibition of CK release were observed but without statistical significance (Figure 3). All antagonists administrated alone had no effect on plasma CK release of ischemia-reperfusion when compared with control group.

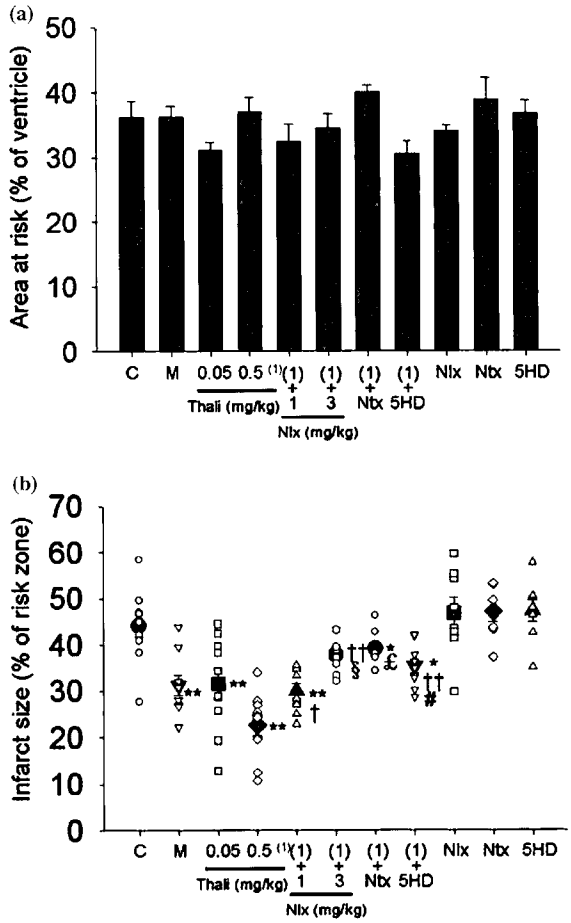

Figure 2. (a) Area at risk (% of ventricle) and (b) infarct size (% of the area at risk) in control rats and in rats treated by morphine, thaliporphine, naloxone, naltrexone, 5HD, thaliporphine plus naloxone, naltrexone or 5HD. Opened symbol indicates infarct size of each individual experimental; closed symbol indicates average value of infarct size of each group. M indicates morphine; Thali indicates thaliporphine; Nlx indicates naloxone; Ntx indicates naltrexone. $*p < 0.05$ vs. control group. $**p < 0.01$ vs. control group. $\dagger p < 0.05$ vs. 0.5 mg/kg thaliporphine group. $\dagger\dagger p < 0.01$ vs. 0.5 mg/kg thaliporphine group. $\S p < 0.05$ vs. naloxone group. $£p < 0.05$ vs. naltrexone group. $\# p < 0.05$ vs. 5HD group.

Tissue MPO activity in ischemia-reperfusion myocardium

To determine the effects of thaliporphine on the accumulation of neutrophil into the ischemia-reperfusion myocardium, we examined myocardial MPO activity in ischemic regions after 2 h reperfusion. In control group, the myocardial MPO activity was 303.8 ± 17.7 U/g tissue. A significant decrease in MPO activity was observed in both

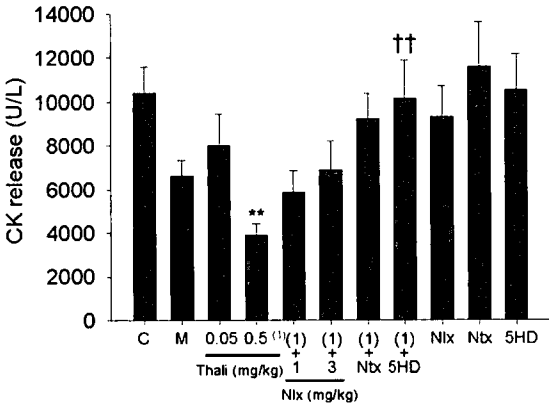

Figure 3. Effects of morphine, thaliporphine, naloxone, naltrexone, 5HD, thaliporphine plus naloxone, naltrexone or 5HD on CK release in anesthetized rat hearts subjected to ischemia (60 min)-reperfusion (120 min). Results are expressed as mean ± s.e. M indicates morphine; Thali indicates thaliporphine; Nlx indicates naloxone; Ntx indicates naltrexone. $**p < 0.01$ vs. control group. $\dagger\dagger p < 0.01$ vs. 0.5 mg/kg thaliporphine group.

thaliporphine (0.5 mg/kg) and morphine treatment groups (132.7 ± 27.5 and 169.4 ± 22.1, $p < 0.01$ vs. control group, respectively). The decrease in cardiac MPO activity by thaliporphine in ischemia-reperfusion myocardium was diminished by pretreatment with naloxone, naltrexone or 5HD (227 ± 17, 255.2 ± 20.5 and 232.6 ± 13.7, $p < 0.05$ vs. 0.5 mg/kg thaliporphine group, respectively). All antagonists administrated alone had no effect on MPO activity in ischemia-reperfusion myocardium when compared with control group (Figure 4).

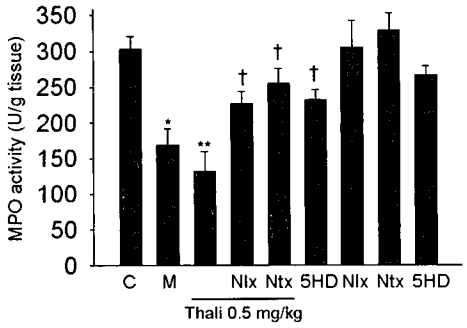

Figure 4. Effects of morphine, thaliporphine, naloxone, naltrexone, 5HD, thaliporphine plus naloxone, naltrexone or 5HD on MPO activity in ischemia-reperfusion hearts. Results are expressed as mean ± s.e. M indicates morphine; Thali indicates thaliporphine; Nlx indicates naloxone; Ntx indicates naltrexone. $*p < 0.05$ vs. control group. $**p < 0.01$ vs. control group. $\dagger p < 0.05$ vs. 0.5 mg/kg thaliporphine group.

Discussion

In the present study, we demonstrate that thaliporphine infused at 10 min before the start of reperfusion decreases plasma CK and infarct size, improves myocardial dysfunction, and reduces myocardial MPO activity in anesthetized rat subjected to cardiac ischemia-reperfusion. This protection can be diminished by the opioid receptor antagonists and the mitochondrial K_{ATP} blocker. Morphine also partially reduces reperfusion injury including decreased infarct size and MPO activity in the myocardium after ischemia-reperfusion. Our findings suggest that through its activation of opioid receptors and subsequent opening mitochondrial K_{ATP} channels, a timely administration of thaliporphine may exert post-ischemic protection. This compound appears to have stronger activity than morphine.

From clinical point of view, agents which reduce infarct size when given before ischemia, but fail to reduce infarct size when given after ischemia are unlikely to be used as clinical adjuncts to prevent reperfusion injury. In a previous study, we have demonstrated that thaliporphine had a powerful cardioprotective effect in rats when given at a dose of 10^{-7} mole/kg (equal to 0.05 mg/kg) before myocardial ischemia [17]. In the present study, however, we found that a ten times higher dose of thaliporphine was necessary to reduce infarct size to a similar degree when given at post-ischemic period. Higher doses might be necessary in the post-ischemic application, compared with the administration before occlusion, since there are no functional coronary collaterals in rats [25] and thus thaliporphine might not reach the ischemic myocardium until the onset of reperfusion. In summary, thaliporphine can be used as a therapeutic adjunct not only in ischemia but also in post-ischemia reperfusion myocardium.

Previous studies have demonstrated that thaliporphine inhibited $CuSO_4$-induced lipid peroxidation in human LDL, scavenged stable nitrogen centered free radical, and superoxide anion [17]. These effects might be beneficial in pathophysiological conditions in which oxyradicals are generated during postischemic reperfusion period [26, 27]. In this study, the post-ischemic cardioprotective effects of thaliporphine in myocardial infarction were also diminished by naloxone. Naloxone, rather than the blockade of myocardial opioid receptors, may produce the inhibition of Na^+ and K^+ channels in cardiomyocytes and contribute to its antiarrhythmic activity [24]. In this study, naloxone administrated at 1 mg/kg did suppress the ischemia-induced arrhythmias. On the other hand, it has been reported that thaliporphine also possess antiarrhythmic activity via blocking Na^+ and K^+ channels [16] which might suggest an interaction with naloxone in this study. However, another opioid antagonist, naltrexone, without antiarrhythmic activity like naloxone, was also found to diminish the effects of thaliporphine in the present study. Although there are no directly evidence on the cardiac electrophysiology of naltrexone, but it is tempting to speculate that naltrexone possess less action on myocardial Na^+ and K^+ channels at the dose we used in the *in vivo* animal model. These observations suggest that the activation of the opioid receptors plays a major role for thaliporphine in preserving the myocardium from reperfusion injury.

Previous studies with morphine have demonstrated an interaction of opioid receptors and K_{ATP} channels in many organ systems. The protective effect of morphine in the myocytes was also mediated via activation of the K_{ATP} channel. Two distinct populations of K_{ATP} channels are known to exist in the heart: the sarcolemmal K_{ATP} channels and the mitochondrial K_{ATP} channels. Increasing evidence suggests that mitochondrial K_{ATP} channel opening is a critical element in the signal transduction that is responsible for opioid-induced cardioprotection against myocardial ischemia-reperfusion [18, 19, 21, 28, 29]. In this study, with the use of the mitochondrial K_{ATP} channel inhibitor 5-HD [30, 31] we demonstrate that post-ischemic cardioprotection of thaliporphine was also via activation of the K_{ATP} channel and suggest that the mitochondrial K_{ATP} channel may play a role in this cardioprotection.

Enhanced post-ischemic function recovery by thaliporphine is unlikely to be mediated through its direct inotropic action on the heart. Su et al. reported that thaliporphine produces a positive inotropic and a negative chronotropic action, via a partial Ca^{2+} channel-activating activity and strong Na^+ and K^+ channel-blocking activities. However the concentration of thaliporphine exerted the positive inotropic effect in isolated myocardial tissues is approximately 10 μM [16]. It is unlikely that the plasma concentration of

thaliporphine in rats can reach this range with the low dose (0.5 mg/kg) used presently.

Recovery of mechanical function after an ischemic-perfusion insult is influenced by both a combination of the number of surviving myocytes and the degree to which they have been stunned [32]. It is tempting to speculate that thaliporphine increases the number of surviving myocytes, because increases ventricular function was found to correlate well with a greater reduction in infarct size.

The administration of morphine at 0.3 mg/kg at 10 min prior to reperfusion reduced cardiac infarction [14]. In the present study, we also found a reduction of cardiac MPO activity by morphine which indicated a decrease of neutrophil infiltration in ischemic–reperfusion myocardium. However, despite the beneficial attenuation of post-ischemic injury, morphine produced little improvement in cardiac function. In comparison, at clinically relevant doses, morphine produces less infarct size reducing capabilities compared to the thaliporphine treatment group. Multifactorial mechanism of thaliporphine in cardioprotection may potentially contribute to increase cardioprotective activity and to display lower acute toxicity (unpublished observations) than morphine.

Reperfusion injury remains an unmet need for patients with acute myocardial infarction [33]. Treatment with thaliporphine at the end of ischemia efficiently reduces cardiac damage induced by reperfusion injury. In additional to its antioxidant activity, the cardioprotective mechanism of thaliporphine is also mediated by stimulating opioid receptor and opening the K_{ATP} channels. The multifactorial mechanism of thaliporphine may afford a promising drug for preventing cardiac injury during reperfusion in humans.

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References

1. Braunwald E. and Kloner R.A., Myocardial reperfusion: a double-edged sword? J. Clin. Invest. 76: 1713–1719, 1985.
2. Hearse D.J. and Bolli R., Reperfusion induced injury: manifestations, mechanisms, and clinical relevance. Cardiovas. Res. 26: 101–108, 1992.
3. Opie L.H., Reperfusion injury and its pharmacologic modification. Circulation 80: 1049–1062, 1989.
4. Mayfield K.P. and D'alecy L.G., Role of endogenous opioid peptides in the acute adaptation to hypoxia. Brain Res. 582: 226–231, 1992.
5. Mayfield K.P. and D'alecy L.G., Delta-1 opioid receptor dependence of acute hypoxic adaptation. J. Pharmacol. Exp. Ther. 268: 74–77, 1994a.
6. Mayfield K.P. and D'alecy L.G., Delta-1 opioid agonist acutely increases hypoxic tolerance. J. Pharmacol. Exp. Ther. 268: 683–688, 1994b.
7. Meerson F.Z., Ustinova E.E. and Orlova E.H., Prevention and elimination of heart arrhythmias by adaptation to intermittent high altitude hypoxia. Clin. Cardiol. 10: 783–789, 1987.
8. Chien S., Oeltgen P.R., Diana J.N. and Salley R.K., Extension of tissue survival time in multiorgan block preparation with a delta opioid DADLE ([D-Ala2D-Leu5]-enkephalin). J. Thorac. Cardiovasc. Surg. 107: 964–967, 1994.
9. Maslov L.N. and Lishmanov Y.B., Change in opioid peptide level in the heart and blood plasma during acute myocardial ischemia complicated by ventricular fibrillation. Clin. Exp. Pharmacol. Physiol. 22: 812–816, 1995.
10. Schultz J.E., Rose E., Yao Z. and Gross G.J., Evidence for involvement of opioid receptors in ischemic preconditioning in rat hearts. Am. J. Physiol. 268: H2157–2161, 1995.
11. Miki T., Cohen M.V. and Downey J.M., Opioid receptor contributes to ischemic preconditioning through protein kinase C activation in rabbits. Mol. Cell Biochem. 186: 3–12, 1998.
12. Bell S., Sack M.N., Patel A., Opie L.H. and Yellon D.M., Delta opioid receptor stimulation mimics ischemic preconditioning in human heart muscle. J. Am. Coll. Cardiol. 36: 2296–2302, 2000.
13. Ryan T.J., Anderson J.L., Antman E.M., Braniff B.A., Brooks N.H., Califf R.M., Hillis L.D., Hiratzka L.F., Rapaport E., Riegel B.J., Russell R.O., Smith Jr. E.E. and Weaver W.D., ACC/AHA guidelines for the management of patients with acute myocardial infarction. A report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines (Committee on Management of Acute Myocardial Infarction). J. Am. Coll. Cardiol. 28: 1328–1428, 1996.
14. Gross E.R., Hsu A.K. and Gross G.J., Opioid-induced cardioprotection occurs via glycogen synthase kinase beta inhibition during reperfusion in intact rat hearts. Circ. Res. 94: 960–966, 2004.
15. Lee S.S. and Yang H.C., Isoquinoline alkaloids from Neolitsea Konishii K. J. Chin. Chem. Soc. Abstr. 39: 189, 1992.
16. Su M.J., Chang Y.M., Chi J.F. and Lee S.S., Thaliporphine, a positive inotropic agent with a negative chronotropic action. Eur. J. Pharmacol. 254: 141–150, 1994.
17. Hung L.M., Lee S.S., Chen J.K., Huang S.S. and Su M.J., Thaliporphine protects ischemia and ischemia-reperfused rat hearts via an NO-dependent mechanism. Drug Dev. Res. 52: 446–453, 2001.
18. Liang B.T. and Gross G.J., Direct preconditioning of cardiac myocytes via opioid receptors and KATP channels. Circ. Res. 25: 1396–1400, 1999.
19. Mcpherson B.C. and Yao Z., Morphine mimics preconditioning via free radical signals and mitochondrial K(ATP) channels in myocytes. Circulation 103: 290–295, 2001.

20. Hung L.M., Chen J.K., Huang S.S., Lee R.S. and Su M.J., Cardioprotective effects of resveratrol, a natural antioxidant from grapes. Cardiovasc. Res. 47: 549–555, 2000.
21. Schultz J.E., Qian Y.Z., Gross G.J. and Kukreja R.C., The ischemia-selective KATP channel antagonist, 5-hydroxydecanoate, blocks ischemic preconditioning in the rat heart. J. Mol. Cell Cardiol. 29: 1055–1060, 1997.
22. Klein H.H., Puschmann S., Schaper J. and Schaper W., The mechanism of the tetrazolium reaction in identifying experimental myocardial infarction. Arch. Pathol. Anat. 393: 287–297, 1981.
23. Kameswara Rao N.S. and Lee S.S., Preparation of thaliporphine and liriofenine from glaucine by treatment with hydrogen bromide. J. Chin. Chem. Soc. 47: 1227–1230, 2000.
24. Hung C.F., Wu M.H., Tsai C.H., Chu S.H., Chi J.F. and Su M.J., Electrophysiological mechanisms for the antiarrhythmic activities of naloxone on cardiac tissues. Life Sci. 63: 1205–1219, 1998.
25. Maxwell M.P., Hearse D.J. and Yellon D.M., Species variation in the coronary collateral circulation during regional myocardial ischemia: a critical determinant of the rate of evolution and extent of myocardial infarction. Cardiovasc. Res. 21: 737–746, 1987.
26. Tosaki A., Drog-lefaix M.T., Pali T. and Das D.K., Effects of SOD, catalase, and a novel antiarrhythmic drug, EGB 761, on reperfusioninduced arrhythmias in isolated rat hearts. Free Radic. Biol. Med. 14: 361–370, 1993.
27. Kasumi Y., Bernie M. and Hearse D.J., Exacerbation of reperfusion arrhythmias by sudden oxidant stress. Circ. Res. 67: 481–489, 1990.
28. Schultz J.E.J., Hsu A.K. and Gross G.J., Morphine mimics the cardioprotective effect of ischemic preconditioning via a glibenclamide-sensitive mechanism in rat heart. Circ. Res. 78: 1100–1104, 1996.
29. Fryer R.M., Hsu A.K., Nagase H. and Gross G.J., Opioid-induced cardioprotection against myocardial infarction and arrhythmias: mitochondrial versus sarcolemmal ATP-sensitive channels. J. Pharmacol. Exp. Ther. 294: 451–457, 2000.
30. Sato T., O'Rourke B. and Marba'n E., Modulation of mitochondrial ATP dependent K^+ channels by protein kinase C. Circ. Res. 83: 110–114, 1998.
31. Hu H., Sato T., Seharaseyon J., Liu Y., Johns D.C., O'Rourke B. and Marba'n E., Pharmacological and histochemical distinctions between molecularly defined sarcolemmal KATP channels and native cardiac mitochondrial KATP channels. Mol. Pharmacol. 55: 1000–1005, 1999.
32. Yellon D.M. and Downey J.M., Preconditioning the myocardium: from cellular physiology to clinical cardiology. Physiol. Rev. 83: 1113–1151, 2003.
33. Yellon D.M. and Baxter G.F., Protecting the ischemic and reperfused myocardium in acute myocardial infarction: distant dream or near reality. Heart 83: 381–387, 2000.