

‘Opioidergic postconditioning’ of heart muscle during ischemia/reperfusion injury

Marcin Kunecki¹, Wojciech Płazak¹, Tomasz Roleder², Jolanta Biernat³,
Tomasz Oleksy³, Piotr Podolec¹, Krzysztof S. Gołba³

¹Department of Cardiac and Vascular Diseases, John Paul II Hospital, Krakow, Poland

²Department of Cardiology, Medical University of Silesia, Katowice, Poland

³Department of Electrophysiology and Heart Failure, Medical University of Silesia, Katowice, Poland

Abstract

Background: *Ischemic preconditioning and postconditioning are the novel strategies of attaining cardioprotection against ischemia/reperfusion (I/R) injury. Previous studies suggested the role of opioid pathway, however the class of opioid receptors responsible for this effect in humans remains unknown. The aim of the study was to assess the influence of opioids on simulated I/R injury outcomes in the human myocardium.*

Methods: *Trabeculae of the human right atrium were electrically driven in organ bath and subjected to simulated I/R injury. Morphine ($10^{-4}M$, $10^{-5}M$, $10^{-6}M$) or δ -opioid receptor agonist DADLE ($10^{-8}M$, $10^{-7}M$, $10^{-6}M$) was used at the time of re-oxygenation. Additional trabecula was subjected to hypoxia protocol only (Control). Contractive force of the myocardium was assessed as the maximal force of a contraction (A_{max}), the rate of rise of the force of a contraction (Slope L) and relaxation as the rate of decay of the force of a contraction (Slope T).*

Results: *Application of morphine $10^{-4}M$ resulted in increase of A_{max} , Slope L and Slope T during re-oxygenation period as compared to Control ($77.99 \pm 1.5\%$ vs. $68.8 \pm 2.2\%$, $p < 0.05$; $45.72 \pm 2.9\%$ vs. $34.12 \pm 5.1\%$, $p < 0.05$; $40.95 \pm 2.5\%$ vs. $32.37 \pm 4.3\%$, $p < 0.05$). Parameters were not significantly different in the lower morphine concentrations. Application of DADLE $10^{-6}M$ resulted in decrease of A_{max} and Slope L as compared to Control ($68.13 \pm 5.5\%$ vs. $76.62 \pm 6.6\%$, $p < 0.05$; 28.29 ± 2.2 vs. $34.80 \pm 3.9\%$, $p < 0.05$).*

Conclusions: *At re-oxygenation, morphine improves systolic and diastolic function of the human myocardium in the dose-dependent manner. Delta-opioid receptor stimulation attenuates systolic function of human heart muscle which remains in contrast to previous reports with animal models of I/R injury. (Cardiol J 2017; 24, 4: 419–425)*

Key words: ischemia reperfusion injury, postconditioning, opioids

Introduction

The restoration of coronary flow is necessary to reduce ischemic myocardial damage and may save some degree of contractile function to the ischemic heart muscle. However, in the early reperfusion period, an additional damage of cardiac tissue has been observed in the mechanism

known as ischemia/reperfusion (I/R) injury which in clinical settings manifests as the decrease of potential benefits of reperfusion. Sequences of brief ischemia periods applied before (preconditioning [IPC]) or after (postconditioning [POC]) the coronary occlusion are well documented to trigger protective mechanisms to the heart muscle against I/R injury. These phenomena are widely

Address for correspondence: Marcin Kunecki, MD, PhD, Department of Cardiac and Vascular Diseases, John Paul II Hospital, ul. Prądnicka 80, 31–202 Kraków, Poland, tel: +48662140164, e-mail: mkunecki@op.pl

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demonstrated in many species in *in vitro* and *in vivo* studies [1–4]. The mechanisms underlying IPC or POC are still not clarified, but strong experimental evidence suggests that opioids may be part of the endogenous cardioprotective response to I/R injury and trigger intracellular enzyme cascades leading ultimately to closure of the mitochondrial permeability transition pore (mPTP) responsible for induction of cell damage [5]. The functional effects of opioids are mediated via activation of respective opioid receptors (OR). Morphine was the first opioid with proven cardioprotective effect [6]. More recently, several investigators have demonstrated in an animal model of I/R injury that beneficial effect is mediated via the δ -OR pathway [7–9]. However, the class of ORs responsible for this effect in humans remains unknown [10]. Bell et al. [11] have shown that OR activation before prolonged hypoxia preserved the force of contraction of isolated human myocardium. The current study was taken to delineate the effect of different doses of OR modulators (non-selective opioid receptor agonist: morphine, selective δ -opioid receptor agonist D-Ala D-Leu–Enkephalin [DADLE]) applied at the reperfusion on the function of human ischemic myocardium.

Methods

The experiments were performed on muscular trabeculae obtained from the right heart atrial appendages of 67 consecutive patients (39 males/28 females) subjected to the coronary artery bypass surgery. Patients diagnosed with the significant valvular heart disease or with severe heart failure therapy were excluded from the study. Mean ejection fraction was $52.3 \pm 2.39\%$. No trabeculae were excluded in this study.

The fragments of the human right heart atria were immediately transported from the cardiac surgery room to the laboratory in an ice-cold Krebs-Henseleit solution ([mmol/L]: NaCl 118.0, KCl 4.70, CaCl₂ 1.52, MgSO₄ 1.64, NaHCO₃ 24.88, KH₂PO₄ 1.18, glucose 11.0, and sodium pyruvate 2.0; pH 7.4). Two muscular trabeculae were dissected from the right heart atria and incubated in two separate organ baths (Schuler Organbath, Hugo Sachs Elektronik, March-Hugstetten, Germany [HSE]) both filled with Krebs-Henseleit solution warmed up to 37°C. To avoid core hypoxia, trabeculae included in the study had a cross-sectional area of less than 1 mm in diameter.

The Local Bioethics Committee approval for the use of human tissue was obtained (NN-

-6501/98/07) and individual patient consent from all patients was waived. All experiments were performed according to the principles stated in the Declaration of Helsinki. Morphine was obtained from Polfa S.A. DADLE was obtained from Sigma Aldrich Co.

Protocols

Two trabeculae from each patient were always studied simultaneously and exposed to hypoxia protocol including: 60 min of hypoxia (incubation in Krebs-Henseleit buffer deprived of glucose and pyruvate, and saturated with 95% argon and 5% carbon dioxide) with subsequent 60 min of re-oxygenation (incubation in Krebs-Henseleit buffer saturated with 95% oxygen and 5% carbon dioxide). The buffer was replaced every 15 min, except the time of hypoxia. To determine the effects of opioid cardioprotection, 3 doses of morphine or DADLE were administered. Morphine (10^{-4} M, 10^{-5} M, or 10^{-6} M) or DADLE (10^{-8} M, 10^{-7} M or 10^{-6} M) were used at the time of re-oxygenation. The second trabecula was subjected only to hypoxia protocol (Control). Additional trabeculae were exposed to 120-min non-hypoxic stimulation in Krebs-Henseleit buffer saturated with 95% oxygen and 5% carbon dioxide (Sham). Every trabecula was stretched to 90% of its optimal tension strength, according to the Frank-Starling relationship and all trabeculae were driven throughout experiments with 1 Hz 50 ms² stimuli using platinum field electrodes and a stimulator (Type 215, HSE). The systolic function of every trabecula was recorded with the use of F30 isometric force transducer (Type 372, HSE). The signal was enhanced with a bridge amplifier (Type 336, HSE) and recorded by a PowerLab/4SP system and analyzed off-line using Chart software (AD Instruments, Chalgrove, Oxfordshire, UK). Each experimental protocol was completed with 10 μ M of norepinephrine (NE) application to assess the viability of trabeculae. The contractive force of the myocardium assessed as the maximal force of a contraction (*A*_{max}), the rate of rise of the force of a contraction (Slope *L*) and relaxation assessed as the rate of decay of the force of a contraction (Slope *T*) was obtained in 5th, 10th, 15th, 30th, 45th and 60th min of re-oxygenation and after the NE application.

Data analysis

The results were presented as the percent of values obtained before experimental protocol application. All continuous data were normally distributed and presented as a mean \pm standard

Table 1. Parameters of function of human myocardium subjected to morphine/DADLE, Sham or Control protocol.

Protocol	N	Amax [%]	Slope L [%]	Slope T [%]
Morphine 10 ⁻⁴ M	9	77.99 ± 1.5	45.72 ± 2.9	40.95 ± 2.5
Control	9	68.8 ± 2.2	34.12 ± 5.1	32.37 ± 4.3
p		< 0.05	< 0.05	< 0.05
Morphine 10 ⁻⁵ M	10	72.78 ± 2.0	40.81 ± 2.3	35.73 ± 2.0
Control	10	68.96 ± 2.0	35.52 ± 2.3	32.30 ± 2.0
p		0.239	0.113	0.153
Morphine 10 ⁻⁶ M	8	68.69 ± 1.4	35.57 ± 1.7	32.14 ± 2
Control	8	68.52 ± 2.1	37.04 ± 2.9	32.47 ± 3.2
p		0.849	0.524	0.884
DADLE 10 ⁻⁶ M	7	68.13 ± 5.5	28.29 ± 2.2	24.50 ± 2.1
Control	7	76.62 ± 6.6	34.80 ± 3.9	28.85 ± 3.7
p		< 0.05	< 0.05	0.487
DADLE 10 ⁻⁷ M	15	61.57 ± 1.6	34.31 ± 1.8	27.14 ± 1.6
Control	15	67.71 ± 2.8	34.29 ± 3.2	30.76 ± 2.7
p		< 0.05	0.812	0.463
DADLE 10 ⁻⁸ M	9	71.74 ± 1.4	35.85 ± 3.3	34.75 ± 3.9
Control	9	75.07 ± 2.5	45.47 ± 5.8	37.53 ± 5.6
p		0.374	< 0.05	0.413
Sham	9	89.25 ± 2.2	87.46 ± 2.5	85.52 ± 3.1

Data are presented as mean ± standard error and compared by two way analysis of variance with Holm-Sidak *post hoc* test. P < 0.05 marked as significantly different from value in Control (intergroup comparison); Amax — maximal force of a contraction; DADLE — D-Ala D-Leu-Enkephalin; Slope L — rate of rise of the force of a contraction; Slope T — rate of decay of the force of a contraction

error (SE). Two-way analysis of variance (ANOVA) followed by Holm-Sidak test was used to compare the results of values from 5th to 60th min of re-oxygenation (SigmaPlot 10.0.1.2). A p value less than 0.05 was considered statistically significant.

Results

There were no significant differences in age, sex and pharmacotherapy between the patients from whom the trabeculae were taken and were subjected to morphine, DADLE, Control protocols.

Application of morphine 10⁻⁴M resulted in increase of Amax, Slope L and Slope T during re-oxygenation period as compared to Control. Parameters were not significantly different in the lower morphine concentration group. Application of DADLE 10⁻⁶M resulted in decrease of Amax and Slope L as compared to Control with no significant differences for Slope T. In the lower DADLE concentration (10⁻⁷M), we observed significant decrease of Amax vs. Control, with no differences for Slope L, Slope T. Application of DADLE 10⁻⁸M resulted in

decrease of Slope L as compared to Control with no significant differences for Amax, Slope T.

All systolic and diastolic parameters were significantly higher for non-hypoxic stimulation (Sham) as compared to morphine, DADLE and Control protocols during re-oxygenation period and after NE application.

All detailed results are included in Table 1 and visualized in Figures 1 and 2.

Discussion

Morphine has received research interest particularly for pain treatment. The European Society of Cardiology guidelines recommend administration of intravenous morphine to relieve pain in acute cardiac infarction [12]. Moreover, according to American Heart Association guidelines, morphine has a beneficial effect on non-ST elevation acute coronary syndrome (NSTEMI-ACS) [13]. Morphine is a part of pulmonary edema treatment due to the anxiolytic and vasodilatory properties. Non-specific depression of the central nervous system is probably an important factor for hemodynamics in

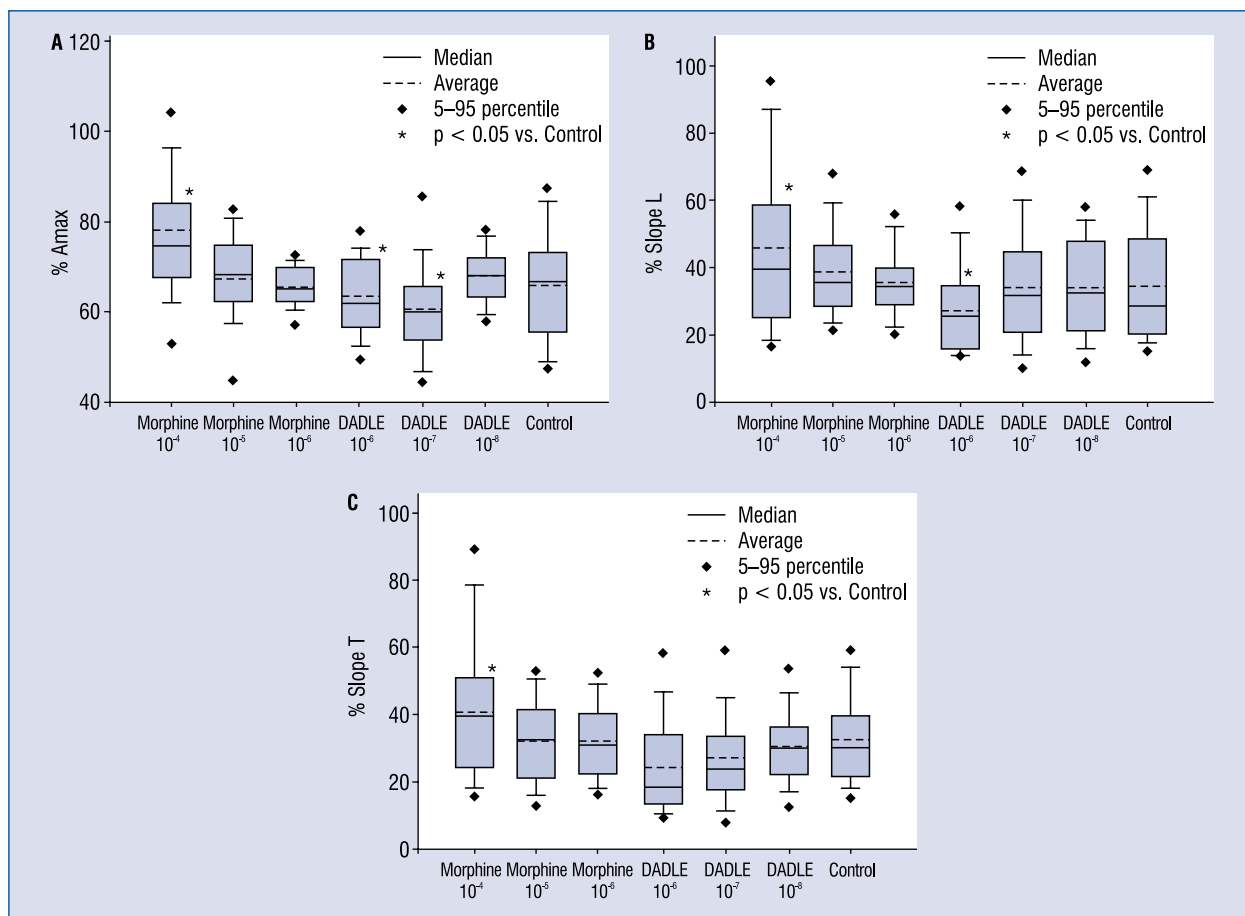


Figure 1. The effect of opioid receptor modulators on function of human myocardium during the re-oxygenation period. Figures present analysis of the contractive function as the maximal force of a contraction (Amax; **A**) and the rate of rise of the force of a contraction (Slope L; **B**), and assessment of the relaxation as the rate of decay of the force of a contraction (Slope T; **C**); *p < 0.05 marked as significantly different from value in Control (intergroup comparison).

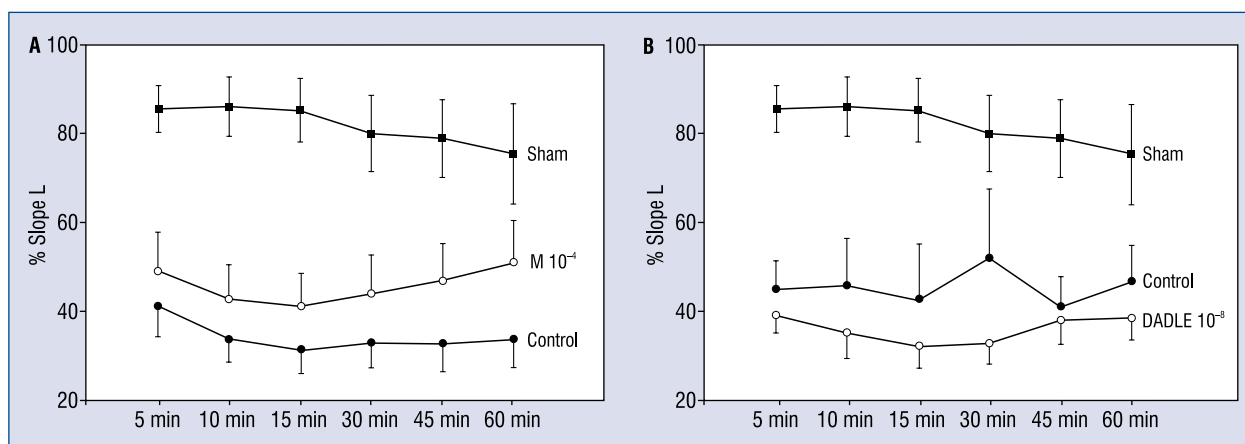


Figure 2. Function of the human myocardium within 5th and 60th min of re-oxygenation period, subjected to morphine (M) 10⁻⁴M (**A**) or D-Ala D-Leu-Enkephalin (DADLE) 10⁻⁸M (**B**) compared with Sham or Control protocols. Figures present parameter of the contractive function of the myocardium — rate of rise of the force of a contraction (Slope L). Sham presented non-hypoxic conditions.

pulmonary edema [14]. Opioids are also commonly used in a perioperative period of coronary artery by-pass grafting (CABG).

The results showed that morphine administered at the onset of reperfusion improved functional recovery in human heart tissue. We observed the maximal protective effect of morphine at a 10^{-4} M concentration. Moreover, we noted that activation of δ -OR led to decrease of myocardial systolic function.

Previous studies on the influence of opioids in human cardiac tissue utilized mainly the OR modulators applied before the lethal hypoxic period [8, 15]. The novelty of our study is that morphine and DADLE were applied at the reperfusion that makes the study protocol compatible with usual clinical circumstances. Our study was performed on isolated fragments of human right atria. For functional studies, atrial tissue sampling can avoid the influence of confounding factors, like the effect of drugs or the presence of collateral circulation. In this model, we did not assess the infarct size as in previous papers, but the differences of contractility as a functional consequences of cardiac ischemia.

Experimental data from multiple species indicate that OR activity is implicated in cardioprotection. However, there is some doubt regarding the OR subtype primarily responsible for the cardioprotective effect.

Beneficial effect of selective δ -OR agonists has been confirmed in a variety of models including rat cardiomyocytes [16] *in situ* and *ex vivo* rat hearts [17], rabbit model [18], or canine [19]. Otherwise, studies with animal models reported that κ -OR but not δ -OR stimulation provided both infarct size limiting and antiarrhythmic effect [20, 21]. More recently, Tsai et al. [22] reported that the protective effect may be caused by the activation of OR signaling pathway with the highest influence of κ -OR and the lowest of μ -OR stimulation. In contrast, some investigators reported in the isolated rat hearts, detrimental influence of κ -OR activation [23]. Based on the previous studies showing that δ -OR and κ -OR, but not μ -ORs were present in rat cardiac tissue [24], investigators concluded that stimulation of δ -OR and κ -OR conferred cardioprotection. Otherwise, beneficial effect of selective μ -OR agonist — remifentanyl in rats remains unclear [25]. Whether this effect is involved in cross-talk with other ORs or roles of extracardiac μ -ORs, remains to be determined. Although, μ -ORs are present in human cardiomyocytes. Indeed, cardioprotective effect of μ -OR stimulation in humans, confirmed in the clinical study with remifentanyl administered in patients subjected to CABG [26].

We presented morphine concentration of 10^{-4} M to be cardioprotective. Lesser concentrations did not show protective effect. According to the previous reports, in the rabbit heart, 3 mg/kg of morphine is required to achieve protective effect [27]. This dose is much higher than clinically used: in humans, the standard dose is about 0.14 mg/kg. Interestingly, administration of helium by inhalation in rabbits can reduce the effective threshold dose of morphine to 100 μ g/kg. Noble gases, through the activation of a RISK kinase pathway, can lead to the closure of mPTP channels during reperfusion period. Moreover, this effect is blocked by naloxone [28]. Similar results was documented with the co-application of morphine and volatile anesthetics like isoflurane [29] and sevoflurane [30]. Further study on cardioprotective effect of inhaled drugs may bring interesting conclusions.

Although many studies are focused on cardioprotective effect of peripheral OR stimulation, there are also several reports outlining that the cardioprotective effects of opioids are mediated through central OR stimulation [31]. Intrathecal infusion of morphine resulted in the reduction of infarct size in rats and the use of naloxone methiodide, not penetrating the brain-blood barrier did not affect the protective effect [32]. Furthermore, protective effect was achieved with morphine in doses of 0.01–1 mg/kg comparable with the effect observed with intravenous administration of morphine in high dose 300 mg/kg [32]. This phenomenon results probably from interaction with central ORs, longer opioid half-life in the cerebro-spinal fluid and slow penetration of the opioid through the blood-brain barrier. Thus, intrathecal opioids allow the use of a lower dose to achieve the similar effect with reduced risk of complications [33].

The differences of species in animal model used in studies might account for the discrepancies in results. In contrast to *in vivo* studies, isolated cardiac tissue has a limited period of biological stability. The viability of the cardiac tissue differs depending on the animal model, for example, in rats, application of 30–40 min of ischemia causes damage to 50% of tissue and in pigs, similar effect is observed after 90 min of ischemia. In our study, due to limited time of heart tissue stability, we analyzed no more than 60 min period after re-oxygenation. In human atrial and ventricular muscle, μ -OR and δ -OR are presented, while κ -OR are negligible. In adult rats, μ -OR are absent. Most of the studies were performed on young animals, whereas intrinsic protective tolerance against I/R injury may fail with age in humans [34, 35].

It was difficult to offer a feasible explanation due to cross-talk between OR-agonists and other receptor/transduction systems. For example, the influence of interleukin 2 (IL-2) on the contraction appears to be mediated via the cardiac κ -OR and the post-receptor signal transduction pathway includes a pertussis toxin sensitive G protein and phospholipase C. This effect in isolated rat heart was completely blocked by κ -OR but no δ -OR antagonists [36]. There are also reports providing evidence that receptors coupled with G protein (GPCRs) form heterodimers resulting in novel ligand binding properties. Furthermore, complexed μ -OR and δ -OR have been shown to be sensitive to ligands selective for δ_2 -OR, as well as all OR types form hetero-oligomers [36]. Moreover, ORs appear to dimerize with other GPCRs, e.g. α_2 - and β_2 -adrenergic receptors [37] or somatostatin receptors [38]. Thus, the OR dimerization results in altered pharmacological characteristics especially associated with ligand binding. In addition, the discrepancies between researches may result from the fact that morphine works in not yet fully understood non-receptor mediated mechanism via modulation of ion channels [35].

Limitations of the study

The results must be interpreted within the limitations of the methodology. The construction of our experiment assumes a control group derived from the same patient and the same factors potentially affecting the test. We must note, however that simulated ischemic model differs from *in vivo* condition. In our experiment, we utilized the buffer, thus there we no elements transporting or binding opioids, like peptides. Based on experiments on animal models of ischemia, we could conclude that administration of δ -OR agonist is sufficient to achieve a cardioprotective effect, with avoiding the side effects of central μ -OR stimulation, e.g. depression of the respiratory center. Our results present detrimental effect of δ -OR agonism. Moreover, in humans, protective effect of opioids might depend on more complicated mechanism than only one type of OR stimulation.

Conclusions

At re-oxygenation, morphine improves systolic and diastolic function of the human myocardium in a dose-dependent manner. Delta-opioid receptor stimulation attenuates systolic function of the hu-

man heart muscle, which remains in contrast to the previous reports with animal model of I/R injury.

Conflict of interest: None declared

References

1. Murry CE, Jennings RB, Reimer KA. Preconditioning with ischemia: a delay of lethal cell injury in ischemic myocardium. *Circulation*. 1986; 74(5): 1124–1136, indexed in Pubmed: [3769170](#).
2. Yellon DM, Alkhalaf AM, Pugsley WB. Preconditioning the human myocardium. *Lancet*. 1993; 342(8866): 276–277, indexed in Pubmed: [8101304](#).
3. Granfeldt A, Lefer DJ, Vinten-Johansen J. Protective ischaemia in patients: preconditioning and postconditioning. *Cardiovasc Res*. 2009; 83(2): 234–246, doi: [10.1093/cvr/cvp129](#), indexed in Pubmed: [19398470](#).
4. Vinten-Johansen J. Postconditioning: a mechanical maneuver that triggers biological and molecular cardioprotective responses to reperfusion. *Heart Fail Rev*. 2007; 12(3-4): 235–244, doi: [10.1007/s10741-007-9024-3](#), indexed in Pubmed: [17520362](#).
5. Lu XH, Ran K, Xiao YY, et al. Protective effects of morphine preconditioning in delayed phase on myocardial ischemia-reperfusion injury in rabbits. *Genet Mol Res*. 2015; 14(3): 8947–8954, doi: [10.4238/2015.August.7.3](#), indexed in Pubmed: [26345826](#).
6. Schultz JE, Hsu AK, Gross GJ. Morphine mimics the cardioprotective effect of ischemic preconditioning via a glibenclamide-sensitive mechanism in the rat heart. *Circ Res*. 1996; 78(6): 1100–1104, indexed in Pubmed: [8635241](#).
7. Peart JN, Gross GJ. Exogenous activation of delta- and kappa-opioid receptors affords cardioprotection in isolated murine heart. *Basic Res Cardiol*. 2004; 99(1): 29–37, doi: [10.1007/s00395-003-0430-y](#), indexed in Pubmed: [14685703](#).
8. Fuardo M, Lemoine S, Lo Coco C, et al. [D-Ala2,D-Leu5]-enkephalin (DADLE) and morphine-induced postconditioning by inhibition of mitochondrial permeability transition pore, in human myocardium. *Exp Biol Med (Maywood)*. 2013; 238(4): 426–432, doi: [10.1177/1535370212474602](#), indexed in Pubmed: [23436882](#).
9. Jang Y, Xi J, Wang H, et al. Postconditioning prevents reperfusion injury by activating delta-opioid receptors. *Anesthesiology*. 2008; 108(2): 243–250, doi: [10.1097/01.anes.0000299437.93898.4a](#), indexed in Pubmed: [18212569](#).
10. Tanaka K, Kersten JR, Riess ML. Opioid-induced cardioprotection. *Curr Pharm Des*. 2014; 20(36): 5696–5705, indexed in Pubmed: [24502571](#).
11. Bell SP, Sack MN, Patel A, et al. Delta opioid receptor stimulation mimics ischemic preconditioning in human heart muscle. *J Am Coll Cardiol*. 2000; 36(7): 2296–2302, indexed in Pubmed: [11127476](#).
12. Steg PG, James SK, Atar D, et al. ESC Guidelines for the management of acute myocardial infarction in patients presenting with ST-segment elevation: The Task Force on the management of ST-segment elevation acute myocardial infarction of the European Society of Cardiology (ESC). *Eur Heart J*. 2012; 33(20): 2569–2619, doi: [10.1093/eurheartj/ehs215](#).
13. Amsterdam EA, Wenger NK, Brindis RG, et al. 2014 AHA/ACC Guideline for the Management of Patients With Non-ST-Elevation Acute Coronary Syndromes: Executive Summary:

- A Report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines. *Circulation*. 2014; 130(25): 2354–2394, doi: [10.1161/cir.0000000000000133](https://doi.org/10.1161/cir.0000000000000133).
14. Ellingsrud C, Agewall S. Morphine in the treatment of acute pulmonary oedema. *Tidsskr Nor Laegeforen*. 2014; 134(23-24): 2272–2275, doi: [10.4045/tidsskr.14.0359](https://doi.org/10.4045/tidsskr.14.0359), indexed in Pubmed: [25492336](https://pubmed.ncbi.nlm.nih.gov/25492336/).
 15. Lemoine S, Zhu L, Massetti M, et al. Continuous administration of remifentanyl and sufentanil induces cardioprotection in human myocardium, in vitro. *Acta Anaesthesiol Scand*. 2011; 55(6): 758–764, doi: [10.1111/j.1399-6576.2011.02456.x](https://doi.org/10.1111/j.1399-6576.2011.02456.x), indexed in Pubmed: [21615344](https://pubmed.ncbi.nlm.nih.gov/21615344/).
 16. Shen H, Ben Q, Zhang Y, et al. Role of $\delta 2$ opioid receptor in cardioprotection against hypoxia-reoxygenation injury. *J Cardiovasc Pharmacol*. 2012; 60(3): 253–261, doi: [10.1097/FJC.0b013e31825e215f](https://doi.org/10.1097/FJC.0b013e31825e215f), indexed in Pubmed: [22592774](https://pubmed.ncbi.nlm.nih.gov/22592774/).
 17. Gross ER, Hsu AK, Gross GJ. Opioid-induced cardioprotection occurs via glycogen synthase kinase beta inhibition during reperfusion in intact rat hearts. *Circ Res*. 2004; 94(7): 960–966, doi: [10.1161/01.RES.0000122392.33172.09](https://doi.org/10.1161/01.RES.0000122392.33172.09), indexed in Pubmed: [14976126](https://pubmed.ncbi.nlm.nih.gov/14976126/).
 18. Peart JN, Gross GJ. Adenosine and opioid receptor-mediated cardioprotection in the rat: evidence for cross-talk between receptors. *Am J Physiol Heart Circ Physiol*. 2003; 285(1): H81–H89, doi: [10.1152/ajpheart.00985.2002](https://doi.org/10.1152/ajpheart.00985.2002), indexed in Pubmed: [12637353](https://pubmed.ncbi.nlm.nih.gov/12637353/).
 19. Peart JN, Patel HH, Gross GJ. Delta-opioid receptor activation mimics ischemic preconditioning in the canine heart. *J Cardiovasc Pharmacol*. 2003; 42(1): 78–81, indexed in Pubmed: [12827030](https://pubmed.ncbi.nlm.nih.gov/12827030/).
 20. Wang G, Wu S, Pei J, et al. Kappa- but not delta-opioid receptors mediate effects of ischaemic preconditioning on both infarct and arrhythmia in rats. *Am J Physiol Heart Circ Physiol*. 2001; 280: H384–H391.
 21. Cheng L, Ma S, Wei LX, et al. Cardioprotective and antiarrhythmic effect of U50,488H in ischemia/reperfusion rat heart. *Heart Vessels*. 2007; 22(5): 335–344, doi: [10.1007/s00380-007-0983-z](https://doi.org/10.1007/s00380-007-0983-z), indexed in Pubmed: [17879026](https://pubmed.ncbi.nlm.nih.gov/17879026/).
 22. Tsai HJ, Huang SS, Tsou MT, et al. Role of Opioid Receptors Signaling in Remote Electrostimulation–Induced Protection against Ischemia/Reperfusion Injury in Rat Hearts. *PLoS One*. 2015; 10(10): e0138108, doi: [10.1371/journal.pone.0138108](https://doi.org/10.1371/journal.pone.0138108), indexed in Pubmed: [26430750](https://pubmed.ncbi.nlm.nih.gov/26430750/).
 23. Aitchison KA, Baxter GF, Awan MM, et al. Opposing effects on infarction of delta and kappa opioid receptor activation in the isolated rat heart: implications for ischemic preconditioning. *Basic Res Cardiol*. 2000; 95(1): 1–10; discussion 11, indexed in Pubmed: [10752540](https://pubmed.ncbi.nlm.nih.gov/10752540/).
 24. Sobanski P, Krajnik M, Shaqura M, et al. The presence of mu-, delta-, and kappa-opioid receptors in human heart tissue. *Heart Vessels*. 2014; 29(6): 855–863, doi: [10.1007/s00380-013-0456-5](https://doi.org/10.1007/s00380-013-0456-5), indexed in Pubmed: [24390763](https://pubmed.ncbi.nlm.nih.gov/24390763/).
 25. Zhang Ye, Irwin MG, Wong TM. Remifentanyl preconditioning protects against ischemic injury in the intact rat heart. *Anesthesiology*. 2004; 101(4): 918–923, indexed in Pubmed: [15448525](https://pubmed.ncbi.nlm.nih.gov/15448525/).
 26. Wong GTC, Huang Z, Ji S, et al. Remifentanyl reduces the release of biochemical markers of myocardial damage after coronary artery bypass surgery: a randomized trial. *J Cardiothorac Vasc Anesth*. 2010; 24(5): 790–796, doi: [10.1053/j.jvca.2009.09.012](https://doi.org/10.1053/j.jvca.2009.09.012), indexed in Pubmed: [20056436](https://pubmed.ncbi.nlm.nih.gov/20056436/).
 27. Ela C, Barg J, Vogel Z, et al. Distinct components of morphine effects on cardiac myocytes are mediated by the kappa and delta opioid receptors. *J Mol Cell Cardiol*. 1997; 29(2): 711–720, doi: [10.1006/jmcc.1996.0313](https://doi.org/10.1006/jmcc.1996.0313), indexed in Pubmed: [9140828](https://pubmed.ncbi.nlm.nih.gov/9140828/).
 28. Pagel PS, Krolikowski JG, Amour J, et al. Morphine reduces the threshold of helium preconditioning against myocardial infarction: the role of opioid receptors in rabbits. *J Cardiothorac Vasc Anesth*. 2009; 23(5): 619–624, doi: [10.1053/j.jvca.2008.12.020](https://doi.org/10.1053/j.jvca.2008.12.020), indexed in Pubmed: [19231239](https://pubmed.ncbi.nlm.nih.gov/19231239/).
 29. Lucchinetti E, da Silva R, Pasch T, et al. Anaesthetic preconditioning but not postconditioning prevents early activation of the deleterious cardiac remodelling programme: evidence of opposing genomic responses in cardioprotection by pre- and postconditioning. *Br J Anaesthesia*. 2005; 95(2): 140–152, doi: [10.1093/bja/aei155](https://doi.org/10.1093/bja/aei155).
 30. Obal D, Dettwiler S, Favocchia C, et al. The influence of mitochondrial KATP-channels in the cardioprotection of preconditioning and postconditioning by sevoflurane in the rat in vivo. *Anesth Analg*. 2005; 101(5): 1252–1260, doi: [10.1213/01.ANE.0000181336.96511.32](https://doi.org/10.1213/01.ANE.0000181336.96511.32), indexed in Pubmed: [16243977](https://pubmed.ncbi.nlm.nih.gov/16243977/).
 31. Weber TP, Stypmann J, Meissner A, et al. Naloxone improves functional recovery of myocardial stunning in conscious dogs through its action on the central nervous system. *Br J Anaesth*. 2001; 86(4): 545–549, indexed in Pubmed: [11573630](https://pubmed.ncbi.nlm.nih.gov/11573630/).
 32. Ling Ling J, Wong GTC, Yao L, et al. Remote pharmacological post-conditioning by intrathecal morphine: cardiac protection from spinal opioid receptor activation. *Acta Anaesthesiol Scand*. 2010; 54(9): 1097–1104, doi: [10.1111/j.1399-6576.2010.02295.x](https://doi.org/10.1111/j.1399-6576.2010.02295.x), indexed in Pubmed: [20887411](https://pubmed.ncbi.nlm.nih.gov/20887411/).
 33. Wong GTC, Ling Ling J, Irwin MG. Activation of central opioid receptors induces cardioprotection against ischemia-reperfusion injury. *Anesth Analg*. 2010; 111(1): 24–28, indexed in Pubmed: [19861363](https://pubmed.ncbi.nlm.nih.gov/19861363/).
 34. Peart JN, Pepe S, Reichelt ME, et al. Dysfunctional survival-signaling and stress-intolerance in aged murine and human myocardium. *Exp Gerontol*. 2014; 50: 72–81, doi: [10.1016/j.exger.2013.11.015](https://doi.org/10.1016/j.exger.2013.11.015), indexed in Pubmed: [24316036](https://pubmed.ncbi.nlm.nih.gov/24316036/).
 35. Headrick JP, See Hoe LE, Du Toit EF, et al. Opioid receptors and cardioprotection - 'opioidergic conditioning' of the heart. *Br J Pharmacol*. 2015; 172(8): 2026–2050, doi: [10.1111/bph.13042](https://doi.org/10.1111/bph.13042), indexed in Pubmed: [25521834](https://pubmed.ncbi.nlm.nih.gov/25521834/).
 36. Cao Z, Liu L, Packwood W, et al. Met5-enkephalin-induced cardioprotection occurs via transactivation of EGFR and activation of PI3K. *Am J Physiol Heart Circ Physiol*. 2005; 288(4): H1955–H1964, doi: [10.1152/ajpheart.00256.2004](https://doi.org/10.1152/ajpheart.00256.2004), indexed in Pubmed: [15563540](https://pubmed.ncbi.nlm.nih.gov/15563540/).
 37. Pepe S, van den Brink OWV, Lakatta EG, et al. Cross-talk of opioid peptide receptor and beta-adrenergic receptor signalling in the heart. *Cardiovasc Res*. 2004; 63(3): 414–422, doi: [10.1016/j.cardiores.2004.04.022](https://doi.org/10.1016/j.cardiores.2004.04.022), indexed in Pubmed: [15276466](https://pubmed.ncbi.nlm.nih.gov/15276466/).
 38. Pfeiffer M, Koch T, Schröder H, et al. Heterodimerization of somatostatin and opioid receptors cross-modulates phosphorylation, internalization, and desensitization. *J Biol Chem*. 2002; 277(22): 19762–19772, doi: [10.1074/jbc.M110373200](https://doi.org/10.1074/jbc.M110373200), indexed in Pubmed: [11896051](https://pubmed.ncbi.nlm.nih.gov/11896051/).