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Ischemic conditioning of human heart muscle depends on opioid-receptor system

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Abstract: Background: Despite progress in the invasive treatment of ischemic heart disease, the ability to limit ischemia-reperfusion (I/R) injury remains largely unrealized. Ischemic pre-conditioning (IPC) and post-conditioning (POC) induce the protective mechanisms of resistance against I/R injury. Stimulation of opioid receptors mimic the protective effect of IPC or POC in an animal models. We tested the hypothesis, that IPC and POC provide cardioprotection in opioid-dependent mechanism in human myocardium.
Methods: Human atrial trabeculae were subjected to I/R injury. To achieve IPC, single hypoxia period preceded the applied lethal hypoxia, to achieve POC triple hypoxia periods followed lethal hypoxia. Naloxone was used at the onset of lethal hypoxia in IPC protocol, and at the time of re-oxygenation in POC protocol. Contractive function of the myocardium was assessed as maximal force of contraction (Amax), rate of rise of force of contraction (+dV/dT) and diastolic parameter — rate of decay of force of contraction (–dV/dT).
Results: Co-application of naloxone with IPC or POC resulted in decrease of Amax, +dV/dT and –dV/dT during re-oxygenation period as compared to IPC or POC only.

C o n c l u s i o n s: Naloxone abrogates beneficial effect of IPC and POC. IPC and POC in humans provide cardioprotection in opioid receptor system dependent mechanism.

Key words: ischemia, naloxone, post-conditioning, pre-conditioning, reperfusion.

Introduction

Ischemic heart disease remains the main cause of morbidity and mortality in developed countries. The restoration of coronary flow is mandatory to reduce ischemic myocardial damage and may save to some degree contractile function to the ischemic heart muscle. However, reperfusion has the potential to exacerbate lethal tissue injury in the mechanism known as ischemia/reperfusion (I/R) injury which in clinical settings manifests as the decrease of potential benefits of reperfusion [1, 2]. Sequences of brief ischemia periods applied before (preconditioning — IPC) or after (post-conditioning — POC) coronary occlusion are well documented to trigger protective mechanisms to the heart muscle against I/R injury [3–7]. The mechanisms underlying IPC or POC are still not clarified, but experimental evidence with an animal models, suggested that opioids may be part of the endogenous cardioprotective response to I/R injury [8, 9].

The current study was the first time taken to delineate the effect of non-selective opioid receptor antagonist, naloxone, on the protective effect evoked with IPC or POC in the human ischemic myocardium.

Materials and methods

The Local Bioethics Committee approval for the use of human tissue was obtained and individual patient consent was waived. All experiments were performed according to the principles stated in the Declaration of Helsinki.

The experiments were performed on muscular trabeculae obtained from the right heart atrial appendages of 58 consecutive patients (35 males/23 females) subjected to coronary artery bypass surgery. Patients diagnosed with significant valvular heart disease or with severe heart failure therapy were excluded from the study.

The fragments of the human right heart atria were transported from the cardiac surgery room to the laboratory in an ice-cold Krebs-Henseleit solution ([M]: 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, each less than 1 mm in diameter, were dissected from the right heart atria and incubated in 2 separate organ baths (Schuler Organbath, Hugo Sachs Elektronik, March-Hugstetten, Germany [HSE]) both filled with Krebs-Henseleit solution warmed up to 37°C.

Table 1. The patients' demographic information and the preoperative pharmacotherapy.

1	Men/Women	35/23
2	Age (years)	62.8 ± 5.7
3	Ejection fraction (mean ± SD)	52.3 ± 2.39%
4a	Diabetes	12
4b	Diabetes with insulin treatment	7
5	Drugs	
5a	Beta-blockers	n = 43 (75%)
5b	Calcium channel blockers	n = 10 (18%)
5c	Angiotensin II converting enzyme inhibitors	n = 30 (52%)
5d	Angiotensin II receptor blockers	n = 2 (3%)
5e	Statins	n = 40 (69%)

Two trabeculae from each patient were always studied simultaneously and exposed to hypoxia protocol including: 60 min of hypoxia (incubation in Krebs--Henseleit buffer deprivated 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 the 95% oxygen and 5% carbon dioxide). The buffer was replaced every 15', except the time of hypoxia.

Protocols

The study protocols are presented in Fig. 1. To achieve IPC, the single brief hypoxia period preceded the applied 60' lethal hypoxia, whereas POC protocol consisted of 3 times repeated sequence of 1-min re-oxygenation with subsequent 1-min hypoxia applied at the beginning of 60 min re-oxygenation. The number of the ischemic cycles was based on previously published data [10, 11]. To determine the effects of opioid receptor blockade on IPC or POC, non-selective opioid receptor antagonist, naloxone (10^{-5}M) was administered at the onset of lethal hypoxia in the IPC protocol, and at the time of re-oxygenation in the POC protocol. The second trabecula was subjected IPC or POC protocol only. 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 ms square 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 μM of norepinephrine (NE) application to assess the viability of trabeculae. The contractive force of the myocardium assessed as the maximal force of contraction (Amax[mV]), the rate of rise of the force of a contraction (+dV/dT) and diastolic parameter — the rate of decay of the force of a contraction (–dV/dT) were obtained in 5th, 10th, 15th, 30th, 45th and 60th min of re-oxygenation and after the NE application.

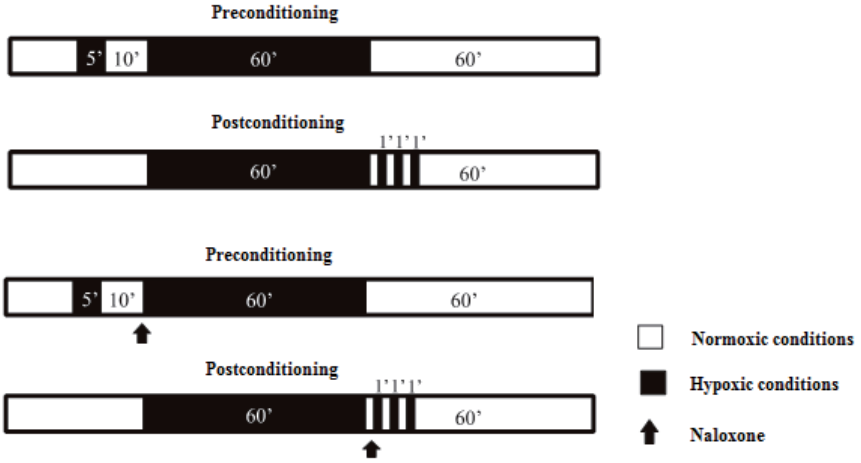

Fig. 1. Protocols for the experimental groups. All protocols were preceded by a stabilization period of 45 to 60 min. This was followed by 60 min of simulated ischemia (superfusion with hypoxic, substrate free Krebs-Henseleit solution and pacing at 1 Hz) and 60 min of superfusion with reoxygenated Krebs-Henseleit solution.

Data analysis

The results were presented as the percent of values obtained before experimental protocol application. All continuous data were normally distributed and were presented as a mean ± standard error of the mean (SEM). Two-way analysis of variance (ANOVA) followed by Holm-Sidack's test was used to compare the results of values from 5th to 60th min of re-oxygenation. A p value less than 0.05 was considered statistically significant. Statistical analysis was performed using SigmaPlot software (ver. 10.0.1.2. Systat Software Inc. San Jose, CA, USA).

Results

There were no significant differences in age, sex and pharmacotherapy between the patients from which the trabeculae were taken and were subjected to IPC, POC or naloxone protocols. The results are presented in Fig. 2.

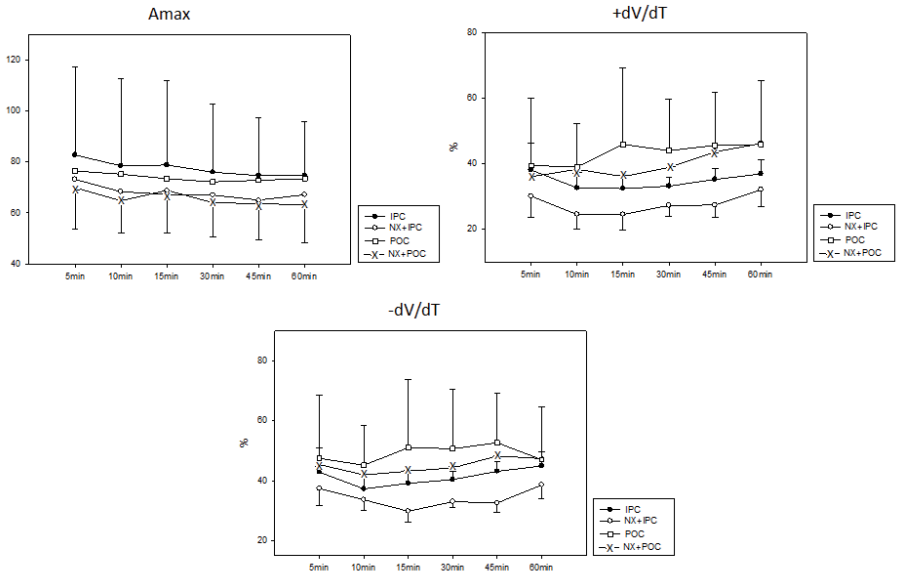

Fig. 2. The effect of ischemic pre-conditioning (IPC), post-conditioning (POC) with/without naloxone (NX) on function of human myocardium during the re-oxygenation period. Figures present analysis of the systolic parameters as maximal force of contraction (Amax), the rate of rise of the force of a contraction (+dV/dT) and diastolic parameter — the rate of decay of the force of a contraction (–dV/dT). The results analysed by two-way ANOVA during the re-oxygenation period.

The co-application of naloxone 10^{-5}M with IPC resulted in the decrease of Amax, +dV/dT and –dV/dT during re-oxygenation period as compared to IPC protocol only.

The results were as follows: naloxone + IPC vs. IPC for Amax, +dV/dT, –dV/dT: 67.99 ± 3.6% vs. 77.54 ± 6.3%*; 27.63 ± 1.9% vs. 34.70 ± 3.4%*; 34.15 ± 1.8% vs. 41.25 ± 3.1%*, respectively, *p <0.05.

The co-application of naloxone 10^{-5}M with POC resulted in the decrease of Amax.

The results were as follows: naloxone + POC vs. POC for Amax, +dV/dT, –dV/dT: 65.73 ± 1.5% vs. 73.90 ± 2.6%*; 39.80 ± 2.1% vs. 43.28 ± 3.6%; 45.05 ± 1.9% vs. 48.97 ± 3.3%, respectively, *p <0.05.

Discussion

Early restoration of coronary artery blood flow following an acute occlusion may limit ischemic heart muscle injury. However reperfusion may itself induce cardiomyocyte death. This phenomenon is known as the ischemia/reperfusion (I/R) injury. The concept of ischemic conditioning include the endogenous cardioprotective effect obtained by applying brief non-lethal episodes of ischemia and reperfusion to the heart [10]. Beneficial effects of cardioprotective strategies: pre- and post-conditioning

have been described in many experimental studies in an animal models of myocardial infarction [11–13]. The number and duration of each phase of hypoxia and reoxygenation is essential but efficiency of the protocols is typically variable. The most effective protocol for human myocardium described in the experimental studies consisted of a 5-minute period of hypoxia and 5 minute reoxygenation period preceding the lethal hypoxia [14], what was presented also in our previous studies [15].

In the current study we used the isolated fragments of human atrial tissue, harvested from patients subjected to cardiac surgery. This model of human heart ischemia provides a possibility to determine the influence of cardioprotective strategies taking into the consideration the all factors potentially affecting the results of the study, like comorbidities or pharmacotherapy. This method allowed us to assess contractile force of myocardium, although ventricular muscle would be preferable. This method allowed us to determine whether the beneficial effect of ischemic preconditioning or post-conditioning is mediated via opioid receptor stimulation in the human heart muscle. The current results showed that non-selective blockade of opioid receptors completely abolished the protective effect of IPC and POC, what has been demonstrated by decreased systolic and diastolic parameters after naloxone administration, comparing with IPC or POC protocols only. Our finding supports the statement that opioid receptor stimulation protects the heart muscle against ischemia/reperfusion injury by mimicking pre- or post-conditioning.

Previous studies on the animal model of heart ischemia have shown that opioid receptors are present in cardiomyocytes. In adult rat heart muscle, δ-, κ-opioid receptors are considered, but μ-opioid receptors are absent [16]. Thus, based on the studies with animal models, the δ- and κ-opioid receptors are implicated in cardioprotection including anti-infarct and anti-arrhythmic effect, although there are few researches presenting results controvert to the previous reports indicating detrimental influence of κ-opioid receptor activation [17]. Unexpectedly, beneficial effect of selective μ-opioid receptor agonist — remifentanil was shown in adult rats [18]. Whether this effect is involved in cross-talk with other opioid receptors or may influence extracardiac μ-opioid receptors, remains to be determined. Whereas, in humans, the presence of all subtypes of opioid receptors in the cardiac tissue was proven with PET imaging [19].

Few clinical trials have provided evidence for beneficial effect of opioids in patients subjected to coronary artery bypass grafting (CABG) or coronary angioplasty. For example remifentanil has been shown to reduce cardiac injury markers in perioperative CABG period [20, 21]. Despite promising results in experimental studies, the main limitation of the IPC is a lack of possibility of application of brief ischemia/reperfusion episodes at the beginning of ischemia or before clinical symptoms. Noda *et al.* presented protective effect of a first pre-infarction angina in patients before developing acute heart infarction, compared with patients who had

a heart infarction as the first manifestation of coronary artery disease, what indicates the existence of the IPC effect in humans [22]. Moreover, post-conditioning may reduce the occurrence of malignant ventricular arrhythmias in patients with STEMI treated with primary coronary angioplasty [23]. Clinical applicability of IPC is limited to cardiosurgical and transplantological procedures. The results of recently published randomized trial ERICCA showed that remote ischemic preconditioning did not improve clinical outcomes in patients undergoing elective on-pump CABG with or without valve surgery [24]. Post-conditioning effect in clinical setting was achieved after percutaneous revascularization by interrupting myocardial reperfusion with six cycles of inflation and deflation of coronary angioplasty balloon. This protocol has been reported to reduce myocardial infarct size at 6 months and preserve the left ventricular ejection fraction at 1 year [25], but large multicenter studies are required to determine the effect of cardioprotective strategies on clinical outcomes. The encouraging results of animal model of cardioprotection are not adequately representative for clinical settings. Lack of strong outcomes dampens the enthusiasm of researchers about clinical application of preconditioning. Hovewer, opioids may mimic the effect of pre- or post-conditioning involving a similar intracellular pathway, or acting in not fully known mechanism [26].

Limitations

The results must be interpreted within the limitations of the methodology. 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, so there we no elements transporting or binding opioids, like peptides. However, the pathophysiological and functional changes that take place in our model of I/R injury are comparable to the change that takes place in in vivo conditions.

Conclusions

Naloxone completely abrogates beneficial effect of both cardioprotective strategies — ischemic pre-conditioning and post-conditioning. These data suggest that ischemic pre-conditioning and post-conditioning in humans provide cardioprotection in opioid receptor system dependent mechanism.

Funding sources

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Conflict of interest

None declared.

List of abbreviations

Amax — maximal force of the contraction
ANOVA — two-way analysis of variance
CABG — coronary artery by-pass grafting
I/R injury — ischemia/reperfusion injury
IPC — ischemic preconditioning
NE — norepinephrine
NX — naloxone
OR — opioid receptor
POC — ischemic postconditioning
SEM — standard error of the mean
STEMI — ST-elevation myocardial infarction
+dV/dT — the rate of rise of the force of a contraction
–dV/dT — the rate of decay of the force of a contraction

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