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The role of protein kinase C and PI3-kinase in the mechanism of the cardioprotective effect of remote ischemic postconditioning

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ABSTRACT

Background. Acute myocardial infarction (AMI) with ST segment elevation is associated with high incidence of complications. Mortality from AMI is about 5%, which has not decreased in recent years. Revascularization provides recovery of coronary blood flow, but also contributes to the occurrence of reperfusion injury to the heart. Remote ischemic postconditioning (RIPostC) is a promising, non-invasive method that can effectively and safely reduce the infarct size.

The aim of the study was to investigate the role of protein kinase C and PI3-kinase in the development of the infarct-limiting effect of remote ischemic postconditioning.

Materials and methods. The study was performed on Wistar rats. Coronary artery occlusion (45 min) and reperfusion (2 h) were performed. The infarct size (IS) and the size of area at risk (AAR) were assessed. RIPostC was modeled by applying tourniquets to the hind limbs in the hip joint immediately after the restoration of coronary blood flow. All inhibitors were administered intravenously 10 min before reperfusion.

Results. In the control group, the IS / AAR ratio was 44%. RIPostC reduced the IS / AAR ratio by about 50%. Preliminary administration of the protein kinase C inhibitor chelerythrine and the PI3-kinase inhibitor wortmannin eliminated the cardioprotective effect of RIPostC.

Conclusion. The mechanism of the infarct-limiting effect of RIPostC is implemented through activation of protein kinase C and PI3-kinase.

Key words: heart, ischemia, reperfusion, remote ischemic postconditioning.

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Участие протеинкиназы С и PI3-киназы в механизме кардиопротекторного эффекта дистантного посткондиционирования

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РЕЗЮМЕ

Введение. Острый инфаркт миокарда (ОИМ) с подъемом сегмента ST отличается высокой частотой осложнений. Смертность от ОИМ составляет около 5% и в последние годы не снижается. Реваскуляризация обеспечивает восстановление коронарного кровотока, но также способствует возникновению реперфузионных повреждений сердца. Дистантное посткондиционирование (ДПост) является многообещающим неинвазивным методом, способным эффективно и безопасно уменьшить размер инфаркта миокарда.

Цель – изучение участия протеинкиназы С и PI3-киназы в реализации инфаркт-лимитирующего эффекта ДПост.

Материалы и методы. Исследование выполнено на 48 самцах крыс линии Вистар. Осуществляли коронарооккклюзию (45 мин) и реперфузию (2 ч). Оценивали размер зоны некроза и зоны риска. Дистантное посткондиционирование моделировали путем наложения жгутов на задние конечности в области тазобедренного сустава сразу послу восстановления коронарного кровотока. Все ингибиторы вводили внутривенно за 10 мин до реперфузии.

Результаты. В контрольной группе отношение зона инфаркта/зона риска (ЗИ/ЗР) составило 44%. Дистантное посткондиционирование уменьшало соотношение ЗИ/ЗР в 1,5 раза. Предварительное введение ингибитора протеинкиназы С хелеритрина или ингибитора PI3-киназы вортманнина устраняло кардиопротекторный эффект ДПост.

Заключение. Механизм инфаркт-лимитирующего эффекта ДПост реализуется через активацию протеинкиназы С, PI3-киназы.

Ключевые слова: сердце, ишемия, реперфузия, дистантное посткондиционирование.

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INTRODUCTION

The mortality rate for acute myocardial infarction (AMI) is about 5% and has not decreased in recent years[1, 2].Revascularization of an infarct-related coronary artery is an important therapeutic intervention for myocardial infarction [3]. However, recovery of coronary blood flow also has adverse consequences manifested through reperfusion injury of the heart affecting the final infarct size and further prognosis [4]. Currently, in clinical practice there are no highly effective drugs for preventing reperfusion injury of the heart [5]. Literature data suggest that remote ischemic postconditioning (RIPostC) is a promising,

non-invasive method to effectively and safely reduce the infarct size and reduce the risk of developing complications.

RIPostC was discovered in 2005 by a research group led by Prof. J. Vinten-Johansen [6]. RIPostC consists in an increase in myocardial tolerance to prolonged reperfusion after exposure to short-term ischemia-reperfusion of another organ at the time of cardiac reperfusion. It was found that RIPostC helps reduce the infarct size by 50% [7]. However, the molecular mechanisms underlying this effect remain poorly understood.

The aim of this study was to investigate the role of protein kinase C and PI3-kinase in the infarction-limiting effect of remote ischemic postconditioning.

MATERIALS AND METHODS

Male Wistar rats (n = 48), weighing 250–300 g, were used in in the study. All procedures related to keeping and using the animals were carried out in accordance with Directive 2010/63/EU of the European Parliament and of the Council adopted of 22 September, 2010 on the protection of animals used for scientific purposes. The study was approved by the Ethics Committee at the Cardiology Research Institute of Tomsk NRMC. All painful procedures were performed on anesthetized animals.

The rats were anesthetized with α-chloralose (60 mg / kg, intraperitoneally) and ventilated with a SAR-830/P ventilator via a tracheostomy tube. Heart rate was recorded using the Data Acquisition Unit MP35. The infarction-limiting effect of RIPostC and its intracellular mechanisms were studied using a model of 45-minute coronary occlusion and 120-minute reperfusion *in vivo* [8]. The quantitative assessment of the myocardium damage was determined by the ratio of the infarct size to the area at risk (IS / AAR) [8]. The area at risk is considered to be a part of the myocardium that was exposed to ischemia during coronary occlusion. RIPostC was modeled by applying tourniquets to the hind limbs in the hip joint, immediately after restoration of coronary blood flow. The time of ischemia and reperfusion for each phase was 3 cycles of 5 minutes.

The experiment used the following pharmacological agents: a protein kinase C inhibitor chelerythrine was injected at a dose of 0.3 mg / kg [9], a PI3-kinase inhibitor wortmannin was administered at a dose of 25 μg / kg [10]. The inhibitors were administered into the femoral vein 10 min before reperfusion (35 minutes after the onset of coronary occlusion). Chelerythrine and wortmannin were dissolved in 0.1 ml of DMSO and then diluted in 0.9 ml of 20% 2-hydroxypropyl-β-cyclodextrin. The animals of the control group were intravenously injected with a mixture of DMSO / 2-hydroxypropyl-β-cyclodextrin. The rats were removed from the experiment 2 hours after the onset of reperfusion by excision of the heart from the thoracic cavity for subsequent staining and determination of the IS / AAR ratio.

The data were statistically processed using the Statistica 13 software. The Mann – Whitney test was used to assess reliability of the results obtained. The data were presented as mean and standard deviation $M \pm SD$. The threshold significance level p was assumed to be 0.05.

RESULTS

After 45-minute coronary occlusion and 120-minute reperfusion, the IS / AAR index in the control group was 44%. The use of RIPostC helped reduce the IS / AAR ratio by 1.5 times (Figure). Therefore, RIPostC increases resistance of the heart to reperfusion injury. Further research was aimed at studying the signaling mechanism underlying the cardioprotective effect of RIPostC. Thus, preliminary administration (10 minutes before reperfusion and RIPostC) of the protein kinase C inhibitor chelerythrine eliminated the infarct-limiting effect of RIPostC. The use of the PI3-kinase inhibitor wortmannin showed a similar effect (Figure).

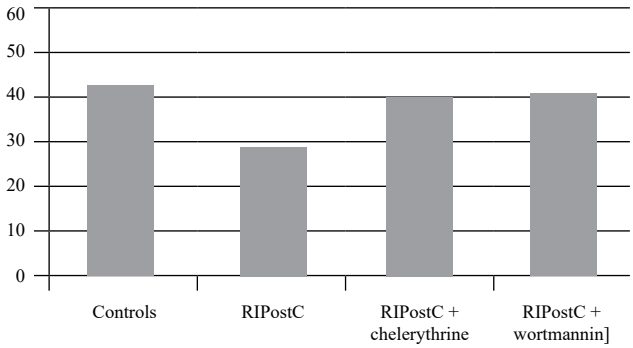

Figure. IS / AAR ratio: *p <0.05 compared to the control

No significant changes in the heart rate were found in the control group during the experiment. No significant changes in the heart rate were observed in the group in which RIPostC was simulated 45 minutes after coronary occlusion (Table). Therefore, the use of RIPostC does not affect the heart rate.

Table

Heart rate values in the model of coronary occlusion and reperfusion, *M* ± *SD*

Group	Observation period				
	Before coronary occlusion	10 minutes before reperfusion	Before reperfusion	30 minutes after reperfusion	2 hours after reperfusion
Control, *n* = 12	364.4 ± 3.5	362.3 ± 3.8	358.7 ± 2.9	353.4 ± 4.3	349.7 ± 5.2
RIPostC, *n* = 12	366.8 ± 3.1	364.8 ± 3.6	363.5 ± 3.2	357.6 ± 4.1	351.5 ± 4.9

DISCUSSION

Literature data suggest that RIPostC has a profound infarct-limiting effect [7]. The results obtained in our research confirmed these data. Literature data indicate that the cardioprotective effect of RIPostC is associated with the activation of protein kinase C, MEK-kinase, and PI3-kinase [7, 11, 12]. Based on these data, we suggested that these kinases can be involved in the mechanism of the infarct-limiting effect of RIPostC. Indeed, according to the results obtained, protein kinase C and PI3-kinase are involved in the mechanism of the cardioprotective effect of RIPostC, which indicated the similarity of the molecular mechanisms of preconditioning and RIPostC.

According to some data, the cardioprotective effect of RIPostC is a consequence of the appearance of a hydrophobic peptide with a molecular weight of 30 kDa in the blood of experimental animals; this substance is released into the blood from ischemic limbs [13]. The researchers suggest that this humoral factor differs from the known endogenous peptides with infarct-limiting effect (opioid peptides, bradykinin) and significantly exceeds them in molecular weight. Several studies showed that RIPostC has a neuroprotective effect [14]. Therefore, it can be asserted that a distinctive feature of the humoral factor is its ability to penetrate the blood – brain barrier. It can be assumed that the cardioprotective effect of RIPostC may be associated with central mechanisms as well. In turn, to date, there is no clear understanding of what molecular mechanisms underlie the effect of RIPostC.

CONCLUSION

The presented data indicate that remote ischemic postconditioning can increase the resistance of the heart to reperfusion injury. The infarct-limiting effect of RIPostC is implemented through the activation of protein kinase C and PI3-kinase.

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