

Receptor mechanism of infarct-limiting effect of adaptation to normobaric hypoxia

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ABSTRACT

The aim of the study was to investigate the involvement of bradykinin, cannabinoid and vanilloid (TRPV1 channel) receptors in the implementation of the infarct-limiting effect of chronic normobaric hypoxia (CNH).

Materials and methods. The study was performed on male Wistar rats ($n = 117$) weighing 250–300 g. Adaptation to CNH was modeled for 21 days at 12% pO₂, 0.3% pCO₂ and normal atmospheric pressure. A day after adaptation of rats to CNH coronary artery occlusion (45 min) and reperfusion (2 h) was performed. In the study the following compounds were used: selective cannabinoid CB1 receptor antagonist rimonabant (1 mg/kg), selective cannabinoid CB2 receptor antagonist AM630 (2.5 mg/kg), selective bradykinin B2 receptor antagonist HOE140 (50 µg/kg), and vanilloid receptor (TRPV1 channel) antagonist capsazepine (3 mg/kg). All antagonists were administered 15 min before coronary artery occlusion.

Results. Adaptation to normobaric hypoxia promoted the formation of the pronounced infarct-limiting effect. The blockade of B2 receptor eliminated the infarct-limiting effect of CNH. Blockade of cannabinoid or vanilloid receptors did not affect the infarct-limiting effect of CNH.

Conclusion. The infarct-limiting effect of CNH depends on the activation of B2 receptor, and the adaptive increase in cardiac tolerance to ischemia/reperfusion does not depend on cannabinoid or vanilloid receptors.

Key words: myocardium, ischemia, reperfusion, receptors, chronic hypoxia.

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Рецепторный механизм инфаркт-лимитирующего эффекта адаптации к нормобарической гипоксии

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РЕЗЮМЕ

Цель исследования – изучение участия брадикининовых, каннабиноидных и ваниллоидных рецепторов (TRPV1-каналов) в реализации инфаркт-лимитирующего эффекта хронической нормобарической гипоксии.

Материалы и методы. Исследование было выполнено на самцах крыс Вистар ($n = 117$) массой 250–300 г. Адаптацию к гипоксии (ННГ) моделировали в течение 21 сут при 12% pO_2 , 0,3% pCO_2 и нормальном атмосферном давлении. Через 1 сут после адаптации у крыс воспроизводили коронароокклюзию (45 мин) и реперфузию (2 ч). В исследовании использовали следующие препараты: селективный антагонист каннабиноидных CB1-рецепторов римонабант (1 мг/кг), селективный антагонист каннабиноидных CB2-рецепторов AM630 (2,5 мг/кг), селективный антагонист брадикининовых B2-рецепторов HOE140 (50 мкг/кг), антагонист ваниллоидных рецепторов (TRPV1-каналов) капсазепин (3 мг/кг). Все антагонисты вводили за 15 мин до коронароокклюзии.

Результаты. Адаптация к нормобарической гипоксии приводила к формированию выраженного инфаркт-лимитирующего эффекта. Блокада B2-рецепторов устраняла инфаркт-лимитирующий эффект ННГ. Блокада каннабиноидных или ваниллоидных рецепторов не влияла на инфаркт-лимитирующее действие ННГ.

Заключение. Инфаркт-лимитирующий эффект ННГ зависит от активации B2-рецепторов, а адаптационное повышение толерантности сердца к ишемии и реперфузии не зависит от каннабиноидных или ваниллоидных рецепторов.

Ключевые слова: миокард, ишемия, реперфузия, рецепторы, хроническая гипоксия.

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INTRODUCTION

It is known that in chronic moderate hypoxia, nonspecific myocardial resistance to damage during ischemia and subsequent reperfusion is formed. However, the pathways of forming myocardial resistance during adaptation to hypoxia remain poorly understood. In particular, the receptor mechanisms of this phenomenon have not been sufficiently studied. Earlier, we found the participation of opioid receptors in the infarct-limiting [1] and cytoprotective [2] effects of adaptation to continuous hypoxia. However, other receptor mechanisms remain unexplored. At the same time, an important role of bradykinin, cannabinoid and vanilloid receptors in the regulation of the heart's tolerance to ischemia/reperfusion during ischemic and remote preconditioning is known [3–7].

The aim of this study was to investigate the participation of bradykinin, cannabinoid, and vanilloid receptors (TRPV1 channels) in the implementation of the infarct-limiting effect of continuous normobaric hypoxia (CNH).

MATERIALS AND METHODS

The study was performed on male Wistar rats ($n = 117$) weighing 250–300 g. Animals of the experimental groups (adapted to hypoxia) were exposed to CNH (12% pO_2 , 0.3% pCO_2) at normal atmospheric pressure in the chamber for 21 days [1]. Monitoring the state of the gaseous medium was carried out using the TCO-IR and OLC 20 sensors (Oldham) and the Bio-Nova-204G4R1 apparatus (NTO Bio-Nova) through the MX32 control unit (Oldham). 24 hours before the start of the experiment, the animals of the experimental group were removed from the hypoxic chamber. Groups of rats of normoxic control were kept under standard vivarium conditions.

Before the coronary occlusion procedure, the animals were anesthetized with α -chloralose (100 mg/kg i.p.). During subsequent manipulations, the animals were subjected to artificial ventilation with atmospheric air, which was carried out using the SAR-830 Series ventilator (Central Wisconsin Engineers Inc., Schofield, USA) through an intubated trachea. To perform coronary occlusion, the chest was opened at

the intercostal space to the left of the sternum, the heart was freed from the pericardium and a ligature was placed on the left descending coronary artery in its upper third for 45 minutes. Reperfusion was performed by releasing the ligature with visual control of the restoration of coronary circulation by hyperemia of the ischemic region [8]. The duration of reperfusion was 2 hours. To determine myocardial infarction size, the ligature, previously placed on the left coronary artery, was again tightened; the isolated heart was washed through the aorta with physiological saline, and stained with 5% potassium permanganate solution. After washing the myocardium with saline, the right ventricle was separated, both ventricles were weighed, the left ventricle was dissected into sections 1 mm thick parallel to the axis of the heart.

Sections of the left ventricle were stained with a 1% solution of 2,3,5-triphenyltetrazolium (37° C, 30 minutes) and fixed for 1 day in a 10% solution of neutral formalin [8]. Slices were scanned (Scanjet G2710), the size of the necrosis zone and the area at risk (ischemia/reperfusion zone) were determined planimetrically using the application software package. The magnitude of the infarct size was expressed as a percentage of the area at risk. The following drugs were used in the

study: selective cannabinoid CB1 receptor antagonist rimonabant (1 mg/kg), selective cannabinoid CB2 receptor antagonist of AM630 (2.5 mg/kg), selective bradykinin B2 receptors antagonist HOE140 (50 µg/kg), and vanilloid receptor (TRPV1 channels) antagonist capsazepine (3 mg/kg). All antagonists were administered 15 minutes before coronary artery occlusion. The choice of doses of pharmacological agents was based on the previous data [9–12].

Statistical data processing was performed using the Statistica 6.0 software (StatSoft, Inc.). The mean value (*M*) and standard error of the mean (*SEM*) were calculated. The significance of differences between groups was determined using the nonparametric Mann – Whitney *U*-test. The critical significance level was taken as $p = 0.05$.

RESULTS

Adaptation to normobaric hypoxia led to the formation of a pronounced infarct-limiting effect, the size of the infarct formed during coronary occlusion-reperfusion, defined as the ratio of the size of necrosis to the risk zone, was 38% less than in non-adapted rats. It should be noted that the hypertrophy of the right ventricle of the myocardium is characteristic of the state of chronic hypoxia (Table 1).

Table 1

Effect of CB1 cannabinoid receptor antagonist rimonabant and CB2 cannabinoid receptor antagonist Am630 on the infarct size after 45-minute coronary occlusion and 120-minute reperfusion in rats adapted to normobaric hypoxia, $M \pm SEM$						
Groups	<i>n</i>	Area of necrosis, mg	Area at risk, mg	Infarct size, AN/AR(%)	Right ventricular weight, mg	Left ventricular weight, mg
Control	12	186.9 ± 14.4	351.3 ± 9.8	53.2 ± 4.8	175.1 ± 11.3	981.7 ± 13.7
Rimonabant (1 mg/kg)	12	185.6 ± 14.4	355.3 ± 9.8	48.0 ± 4.8	170.1 ± 10.3	981.7 ± 13.7
AM630 (2.5 mg/kg)	12	181.7 ± 19.4	367.1 ± 22.4	49.5 ± 7.2	173.4 ± 10.7	947.8 ± 64.6
CNH	12	124.1 ± 12.8*	369.3 ± 15.1	33.6 ± 6.8*	226 ± 12.2*	958.3 ± 12.6
CNH + rimonabant (1 mg/kg)	12	131.8 ± 13.5*	374.3 ± 17.1	35.2 ± 8.3*	225.3 ± 17.6*	989.3 ± 15.5
CNH + AM630 (2.5 mg/kg)	12	118.6 ± 13.5*	364.8 ± 17.1	32.5 ± 8.2*	233.7 ± 13.9*	969.8 ± 12.9

Note: * $p < 0.05$ compared with the control group, Mann – Whitney *U*-test. AN – area of necrosis, AR – area at risk (here and in Table 2).

It was found that the inhibition of cannabinoid CB1 receptors by the selective antagonist rimonabant did not lead to a change in the infarct size in rats adapted to CNH (Table 1). These data indicate that CB1 cannabinoid receptors are not involved in the formation of the infarct-limiting effect of CNH. The injection of the selective CB2 cannabinoid receptor antagonist AM630 also did not affect the infarct size

during coronary artery occlusion and reperfusion in rats adapted to CNH (Table 1). The administration of the selective cannabinoid receptor antagonists to non-adapted rats did not lead to a change in the infarct size during the subsequent coronary occlusion (Table 1). These data suggest that CB1 and CB2 cannabinoid receptors are not involved in the infarct-limiting effect of CNH.

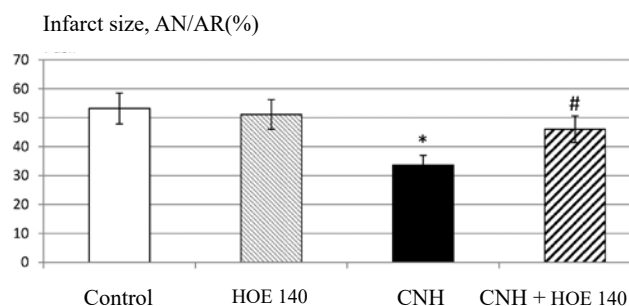


Fig. 1. Effect of the bradykinin receptor antagonist HOE 140 on the infarct size in rats after CNH

Note: * $p < 0.05$ compared with the control group, # – with the CNH group, Mann – Whitney U -test. AN – area of necrosis, AR – area at risk.

Blockade of bradykinin receptors by the selective antagonist HOE140 contributes to an increase in the infarct size in rats adapted to CNH (Fig. 1). Moreover, in non-adapted rats, blockade of the bradykinin receptors did not affect the infarct size. These data indicate that bradykinin receptors are involved in the formation of the infarct-limiting effect of CNH.

Inhibition of vanilloid receptors (TRPV1 channels) by the selective blocker capsazepine did not affect the infarct size in rats after a course of CNH or in non-adapted animals (Table 2). The obtained data allow us to conclude that there is no connection of TRPV1 channel activation and the formation of cardioprotection during adaptation to normobaric hypoxia.

Table 2

Effect of vanilloid receptor (TRPV1 channel) blocker capsazepine on rat infarct size after CNH, $M \pm SEM$						
Groups	<i>n</i>	Area of necrosis, mg	Area at risk, mg	Infarct size, AN/AR(%)	Right ventricular weight, mg	Left ventricular weight, mg
Control	12	186.9 ± 14.4	351.3 ± 9.8	53.2 ± 4.8	175.1 ± 11.3	981.7 ± 13.7
Capsazepine (3 mg/kg)	9	171.9 ± 18.5	353.6 ± 17.1	48.6 ± 6.2	199.4 ± 13.6	917.5 ± 19.3
CNH	12	124.1 ± 12.8*	369.3 ± 15.1	33.6 ± 6.8*	226 ± 12.2*	958.3 ± 12.6
CNH + capsazepine (3 mg/kg)	12	128.9 ± 12.2*	374.3 ± 17.1	34.7 ± 6.1*	218.1 ± 16.1*	976.6 ± 12.5

Note: * $p < 0.05$ compared with the control group, Mann – Whitney U -test. AN – area at necrosis, AR – area at risk.

DISCUSSION

The problem of myocardial protection in ischemic damage remains relevant, despite significant progress in this area. The reason for this is the lack of effective cardioprotective drugs that do not have strong side effects. Currently, beta-blockers, alpha-2-adrenoreceptor agonists, calcium channel blockers, nitrates, statins, and macroergic compounds are proposed to protect the myocardium from ischemic-reperfusion injury [13]. The effectiveness of a number of these drugs is insufficient for anti-ischemic protection, which, in the presence of many side effects, casts doubt on the feasibility of the use of the indicated drugs. Thus, the search for new means for myocardial protection during ischemic-reperfusion exposure remains an urgent task of modern pharmacology.

One of the ways of a directed search for such agents is a study of the mechanisms of non-specific adaptive resistance of the myocardium to ischemic damage. Thus, it is known that the myocardium of animals subjected to moderate chronic hypoxia is more resistant to ischemic effects than the myocardium of intact animals [1, 8, 14]. A study of this phenomenon has been conducted for 60 years, but many aspects of the formation of adaptive myocardial stability remain

unexplored, its receptor mechanisms remain poorly understood. Previous studies in our laboratory have shown participation of opioid receptors in adaptive cardioprotection [1, 2].

This work revealed that bradykinin receptors are also involved in the mechanism of triggering the defense mechanism when adapting to chronic hypoxia. Both types of these receptors are known to be associated with Gi/o proteins that are located on the membrane of cardiomyocyte. Successive activation of receptors (opioid or bradykinin) and Gi/o proteins triggers an intracellular kinase mechanism that turns off protein kinase C, NO-synthase, tyrosine kinase, and subsequently activates ATP-sensitive potassium channels of mitochondria [14]. The result of the latter is inhibition of the opening of the pore that regulates the permeability of mitochondria (MPTP), an increase in the resistance of mitochondria to calcium ions, an improvement in the energy metabolism of mitochondria, and thus a decrease in the sensitivity of cells to the damaging effects of ischemia and reperfusion [15].

CONCLUSION

The obtained results allow us to present bradykinin receptors as one of the key mechanisms for the

formation of the infarct-limiting effect of continuous normobaric hypoxia. Taking into account the data on the important role of opioid receptors in cardioprotection in CNH [1, 2], we can talk about the implementation of the infarct-limiting effect of chronic hypoxia through Gi/o-protein-coupled opioid and bradykinin receptors. Cannabinoid receptors and TRPV1 channels do not participate in the infarct-limiting effect of adaptation to normobaric hypoxia.

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