REVIEW ARTICLE

# Trigger, Signaling Mechanism and End Effector of Cardioprotective Effect of Remote Postconditioning of Heart

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**Abstract:** The hypothetical trigger of remote postconditioning (RPost) of the heart is the high-molecular weight hydrophobic peptide(s). Nitric oxide and adenosine serve as intermediaries between the peptide and intracellular structures. The role of the autonomic nervous system in RPost requires further study. In signaling mechanism RPost, kinases are involved: protein kinase C, PI3, Akt, JAK. The hypothetical end effector of RPost is aldehyde dehydrogenase-2, the transcription factors STAT, Nrf2, and also the $BK_{Ca}$ channel.

## 1. INTRODUCTION

Hospital mortality in patients with ST-segment elevation myocardial infarction (STEMI) in the USA is 4.7% [1]. Primary percutaneous coronary intervention provides > 95% of recanalization of the infarct-related coronary artery [2], however, patients die. One of the causes of death is reperfusion injury of the heart. Drugs which have been approved for clinical use and are capable of preventing reperfusion injury of the heart with high efficacy are not currently available. In our opinion, invaluable help in the search for such drugs could be given by the study of trigger mechanisms of remote postconditioning (RPost). Remote postconditioning of the heart is commonly referred to as increased myocardial tolerance to prolonged reperfusion after exposure to short-term ischemia-reperfusion to another organ at the time of cardiac reperfusion.

The phenomenon of RPost was discovered in 2005 by a group of physiologists from Atlanta (USA), headed by Prof. J. Vinten-Johansen [3]. It is believed that the study of molecular mechanisms, RPost can serve as a basis for the development of fundamentally new drugs that can prevent reperfusion injury of the heart [4-12].

## 2. EXPERIMENTAL DATA ON THE CARDIOPROTECTIVE EFFECT OF RPost

### 2.1. Infarct-reducing Effect of RPost

The phenomenon of remote postconditioning was discovered in 2005 by Professor Vinten-Johansen's group (Fig. 1) [3]. In rats, the researchers performed coronary artery occlusion (30 min) and reperfusion (3 h). Those rats constituted the control group. RPost was modeled using occlusion (5 min) of the renal artery, which was performed 24 min after the ligation of the coronary artery. The kidney reperfusion was started 1 minute before the removal of the ligature from the coronary artery. The third group, which consisted of rats with continuous renal artery occlusion, was ligated 24 minutes after the simulation of experimental myocardial infarction. The fourth group consisted of rats with 5-min ischemia and reperfusion of the kidney 26 min after coronary occlusion. In the latter case, the reperfusion of the kidney was started 1 min after the restoration of coronary blood flow. This group of authors was designated as a group of delayed remote postconditioning [3]. The infarct size was calculated as the infarct size/area at risk (IS/AAR) ratio. The area at risk zone is usually denoted the ischemia-reperfusion area. It turned out that RPost contributes a decrease in the IS/AAR by 50% and at the same time the plasma creatine kinase (CK) level decreased by almost 50%. The continuous renal ischemia did not affect the IS/AAR ratio. This data can be considered as an indirect evidence that the humoral fac-

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tors, which were released from the kidneys during ischemia-reperfusion, have a cardioprotective effect. Delayed RPost had no infarct-limiting effect [3]. Consequently, the cardioprotective humoral factor (s) must have entered the bloodstream before the restoration of coronary perfusion. Similar data was obtained by Gao *et al*. [13]. In that study on rats with coronary artery occlusion (45 min) and reperfusion (3 h), they showed that RPost, which was carried out 15 min after the onset of cardiac ischemia, contributes to a 2-fold reduction in the IS/AAR ratio. Delayed RPost, which was modeled 10 min after cardiac reperfusion, did not affect the IS/AAR ratio.
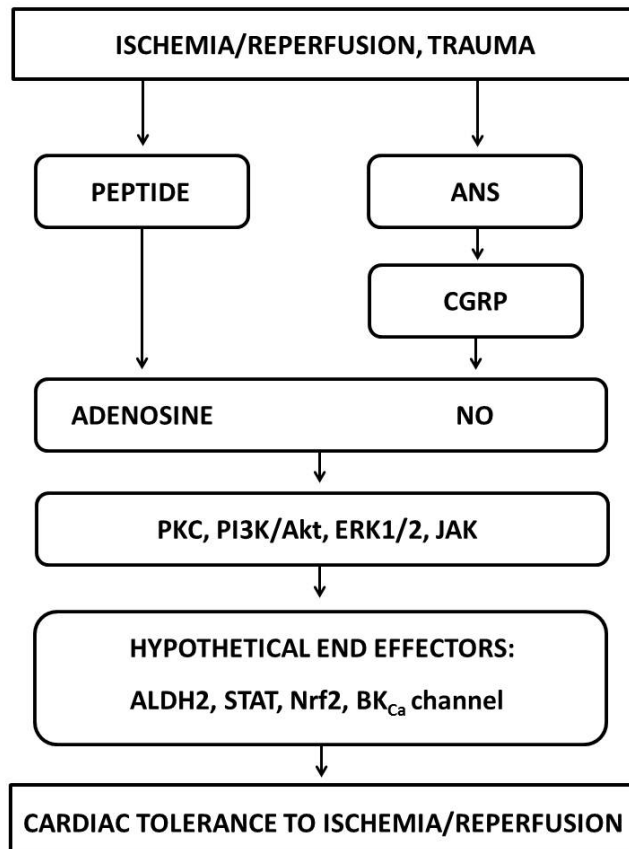


**Fig. (1).** The hypothetical mechanism of infarct reducing effect of remote postconditioning. ANS, autonomic nervous system; CGRP, calcitonin gene related peptide; PKC, protein kinase C; PI3K, phosphatidylinositol-3-kinase; ERK1/2, extracellular signal-regulated kinase; JAK, Janus kinase; Akt, anti-apoptotic kinase; STAT, signal transducer and activator of transcription; ALDH2, aldehyde dehydrogenase-2; Nrf2, Nuclear factor (erythroid-derived 2)-like 2; $BK_{Ca}$ channel, $Ca^{2+}$ dependent big conductance $K^+$ channel.

In 2006, the results of Vinten-Johansen's group were confirmed by Li *et al*. [14], who performed the experiments on rabbits with coronary artery occlusion (30 min) and reperfusion (3 h). The control group included rabbits, which were subjected only to ischemia and reperfusion of the heart. The preconditioning group consisted of animals in which the short-term coronary occlusion (5 min) and reperfusion (5 min) were carried out before a prolonged ischemia-reperfusion. Local postconditioning was performed using 3

cycles of reperfusion (30 s) and ischemia (30 s) after a 30-minute coronary artery occlusion.

Remote postconditioning was modeled by occlusion (5 min) of the femoral artery, which was performed 24 min after coronary artery ligation. The reperfusion of the lower limb was started 1 min before the restoration of coronary blood flow [14]. It turned out that all of the three adapting effects had a practically identical infarct-limiting effect. In addition, they have equally contributed to the decrease in the plasma CK level. In 2007, Andreka *et al*. [15] published the results of their experiments on pigs with coronary occlusion (90 min) and reperfusion (72 h). Infarction size was assessed using serial determination of CK for 72 h, magnetic resonance imaging (MRI), and morphologically as the IS/AAR ratio. Postconditioning was performed before cardiac reperfusion using 4 cycles of inflation (5 min) and deflation (5 min) of an intra arterial balloon which was introduced into the femoral artery. According to the serial determination of CK, the infarct size after postconditioning decreased by 26%, and according to MRI data, it decreased by 22%. The morphological study showed that RPost contributes to a decrease in the IS/AAR ratio by almost by 50% [15]. In 2009, Fang *et al*. [16] reported on intra-cardiac RPost. In the experiments on rats, they performed occlusion (30 min) and reperfusion (3 h) of the left descending coronary artery (LDCA, control group). The infarct size was assessed as the IS/AAR ratio, and the pumping function of the heart was determined using a catheter that was inserted into the cavity of the left ventricle (LV). Remote postconditioning was induced using three sessions of occlusion (10 s) and reperfusion (10 s) the circumflex branch. Local postconditioning was performed using 3 cycles of reperfusion (10 s) and occlusion (10 s) of LDCA. In the control, the IS/AAR ratio was 49%, in the RPost group it was 32%, in the post conditioned rats it was 26%. The authors have failed to detect a noticeable improvement in the pumping function of the heart in both animals with local postconditioning and in rats with RPost [16]. The CK activity in the blood plasma of the control animals was 3.3 U/l, in the RPost group it was 2.0 U/l, in the post conditioned rats it was 2.2 U/l. According to electron microscopy, damage to the mitochondria in the area at risk was less pronounced in both groups of the post conditioned rats [16]. These facts suggest that transient ischemia and reperfusion of one part of the heart can provide an increase in the tolerance of another part of the heart to a long-term ischemia/reperfusion (I/R), apparently due to the appearance of cardioprotective humoral factor (s) in the bloodstream. In 2009 G. Gritsopoulos *et al*. [17] published the results of their experiments on rabbits with occlusion (30 min) and reperfusion (3 h) LDCA. These animals constituted the control group. Local postconditioning was carried out using 4 cycles of reperfusion (1 min) and ischemia (1 min) of LDCA after prolonged ischemia. Remote postconditioning was performed using 4 cycles of ischemia (1 min) and reperfusion (1 min) of the left carotid artery, starting 30 s before the restoration of coronary perfusion. Local RPost was reproduced using 4 cycles of ischemia (1 min) and reperfusion (1 min) of the coronary artery (the authors do not give the name of this artery, but they emphasize that it was not LDCA). In the control animals, the IS/AAR ratio was 47%, in the local RPost group it was 17.7%, in the remote

postconditioning group  this index was 18.4% [17]. In the group of local postconditioning, the IS/AAR ratio was 33%. The authors have concluded that RPost has a more pronounced infarct-reducing effect than the local postconditioning [17]. Surgeons tried to establish how postconditioning and RPost will affect the efficiency of heart transplantation [18]. The experiments were performed on Fischer 344 rats. The rats that had been transplanted without postconditioning were used as a control group. Postconditioning was induced by clamping (15 s) of the aorta and v. cava immediately after heart transplantation, followed by myocardial reperfusion (15 s). This procedure was repeated 3 times. Remote post-conditioning was caused by occlusion (5 min) of the renal artery immediately before the restoration of transplant perfusion. 24 h after the transplantation, the transplanted rat hearts were used for morphological examination. The authors have shown that both postconditioning and RPost contribute to the reduction of histological signs of myocardial damage. According to a morphological study, RPost contributed to a decrease in the intensity of cardiomyocyte apoptosis, while postconditioning did not affect the programmed death of cardiac cells [18]. The authors have evaluated the free radical processes by carbonylation of myocardial proteins. At the same time, the intensity of free radical oxidation of proteins in the myocardium decreased after RPost, local postconditioning did not affect this process [18]. The presented data shows that remote postconditioning is a more effective way to protect a transplanted heart from I/R injury as compared to local postconditioning. In 2011, the same group of investigators [19] published the data of their experiments on rabbits with coronary occlusion (30 min) and reperfusion (3 h). The control group consisted of animals in which only I/R of the heart were performed. Remote post-conditioning was performed using occlusion (5 min) and reperfusion (5 min) of the left pulmonary artery. Cardiomyocyte necrosis was assessed by serum CK level after 3 h from the start of reperfusion. It turned out that RPost contributes to a decrease in the serum CK level in rabbits.

In 2012, M. Basalay *et al.* [20] published the results of their experiments on the rats with coronary artery occlusion (30 min) and reperfusion (2 h). Remote conditioning was performed using occlusion of the femoral artery (15 min): (1) 25 min before coronary occlusion, (2, 3) 10 or 25 min from the onset of ischemia, and (4, 5) 10 or 30 min from the start of reperfusion (delayed RPost). The used conditioning contributed to a decrease (p <0.001) of the IS/AAR ratio by 54%, 56%, 56% and 48% (for the first 4 groups, respectively) compared to the control (only I/R). The presented data indicates that a delayed RPost in a 10-min reperfusion has an infarct-sparing effect [20]. The results of this study contradict the above data from F. Kerendi *et al.* [3] and Gao *et al.* [13], who could not detect the infarct-limiting effect on delayed RPost. The reason for this controversy remains unclear.

The infarct-reducing effect of RPost has been confirmed in other studies [21-23].

However, not all of the investigators were able to confirm the infarct-limiting effect of RPost. In 2013, Zhang *et al.* [24] published the results of their study on the rats with coronary artery occlusion (30 min) and reperfusion (2 h).

Remote postconditioning was induced using single I/R of both hind limbs. It was found that RPost contributed to an increase in the IS/AAR ratio, an increase in the serum level of markers of cardiomyocyte necrosis (CK-MB, troponin-I) compared with the control group (only I/R).

Thus, most studies have shown that remote postconditioning has a pronounced infarct-reducing effect.

## 2.2. Antiapoptotic Effect of RPost

In 2011, Tang *et al.* [19] reported the results of a study on rabbits with a 30-min coronary occlusion and subsequent reperfusion (3 h). In the control group, there were rabbits, in which only I/R of the heart were performed. Remote post-conditioning was performed by occlusion (5 min) and reperfusion (5 min) of the left pulmonary artery. Apoptosis was assessed by the number of TUNEL-positive cells (Terminal deoxynucleotidyl transferase-mediated UTP nick end-labeling, UTP - uridine triphosphate). It was found that RPost contributed to reducing the number of TUNEL-positive cells by 50% compared with the control group [19]. Therefore, RPost can effectively prevent the reperfusion apoptosis of cardiomyocytes. In 2012, Ran *et al.* [22] published the results of experiments on rabbits with coronary occlusion (60 min) and reperfusion (2 h). Apoptosis was assessed by the number of TUNEL-positive cells. The apoptotic index was calculated as the percentage of apoptotic cells of the total number of cardiomyocytes. In the control (I/R), this indicator was 36%, in the RPost group was 22%. In addition, the investigators have determined the expression of the pro-apoptotic protein Bax (Bcl-2-associated X-protein) and the anti-apoptotic protein Bcl-2 (B-cell lymphoma protein-2). Ischemia-reperfusion have resulted in an increase in the amount of these proteins in myocardial tissue. Remote postconditioning contributed to a decrease in the Bax/Bcl-2 ratio, which could contribute to a decrease in the intensity of apoptosis. In addition, the authors found that RPost contributes to a decrease in the activity of the key enzyme of apoptosis, caspase-3, that undoubtedly affects the intensity of apoptosis [22].

Similar data was obtained by Zhu *et al.* [23]. The researchers demonstrated that RPost in rats with coronary occlusion (3 min) and reperfusion (3 h) contributed to an increase in the Bcl-2/Bax index [23]. Similar data was obtained by Yu *et al.* [21]. The expression of proteins Bcl-2 and Bax, they were assessed by the level of mRNA encoding these proteins. It turned out that RPost in rats with experimental myocardial infarction contributes to an increase in the Bcl-2/Bax ratio, at the same time a decrease in the activity of caspase-3 was recorded [21].

Thus, the presented data indicates that remote postconditioning contributes to the suppression of cardiomyocyte reperfusion apoptosis by reducing the activity of caspase-3, enhancing the expression of Bcl-2 protein and reducing the expression of Bax protein.

## 2.3. Autophagy of Cardiomyocytes and RPost

Autophagy is the process of removing of the damaged organelles, such as mitochondria and the sarcoplasmic reticulum [25]. Autophagy provides the cell with nutrients

when they are deficient. Common autophagy leads to cell death. Morphologically, it is detected by the presence of numerous autophagic vacuoles (autophagosomes) [26]. The process of autophagy includes the induction, the formation of autophagosomes, the merging of autophagosomes with lysosomes and the degradation of autophagolysosomes [25]. The activation of autophagy during I/R enhances the expression of beclin-1, a protein recognized as one of the most important triggers of autophagy [25, 27]. In recent years, an opinion has been formed on the favorable effect of autophagy on the course of I/R injury to the heart, on autophagy as a positive process compared with necrosis and apoptosis [25]. Thus, in a number of publications, it was shown that the damage to cardiomyocytes is aggravated by inhibiting the initiation of autophagy in the ischemic phase [28, 29]. It was found that the suppression of beclin-1 expression reduces the I/R activation of autophagy and increases cell death [25] that indicates athe beneficial role of autophagy.

The first paper on the role of autophagy in RPost was published in 2014 [30]. The study was performed on mice with coronary occlusion (30 min) and reperfusion (1 h, 2 h, and 3 h). Remote postconditioning was carried out using three cycles of ischemia (5 min) and reperfusion (5 min) of the left hind limb immediately after the restoration of the coronary blood flow. The investigators have demonstrated that RPost has an infarct-sparing effect, enhances the expression of beclin-1, changes the production of other proteins involved in autophagy (sequestosome-1, LC3-II, LC3-I from microtubule-associated proteins light chain 3). Preliminary intraperitoneal administration of an autophagy inhibitor 3-methyladenine completely eliminated the cardioprotective effect of RPost [30]. These facts suggest that the infarct-limiting effect of RPost is associated with increased autophagy.

### 2.4. Postinfarction Remodeling and RPost

In 2011, a group of investigators [31] tried to find out whether RPost could prevent post-infarction cardiac remodeling. The study included rats with coronary occlusion (45 min) and reperfusion. The infarct size was evaluated 4 and 28 days after coronary artery ligation. The RPost group included rats in which, after 25 min after coronary artery occlusion, a tourniquet was applied to the hind limb for 5 min, then reperfusion of the hindlimb followed (5 min). Used 4 cycles of RPost, a similar effect was called a single RPost. Subsequently, in some rats, RPost was performed every day, in others, 1 time in 3 days. Evaluation of the IS/AAR ratio 4 days after coronary occlusion showed that a single remote postconditioning, daily RPost and RPost once every 3 days provided a decrease in this indicator by about 30%. The same effect was demonstrated 28 days after simulating I/R [31]. Experimental myocardial infarction on the 28th day after ligation of the coronary artery caused LV hypertrophy. It was a myocardial infarction, which was formed after 45-minute ischemia and reperfusion. A single remote postconditioning contributed to a decrease in the degree of cardiac hypertrophy, but an even more pronounced decrease in hypertrophy was observed in animals with multiple RPost. 28 days after coronary occlusion, LV dilatation was recorded. A single RPost also significantly reduced LV dilation, but a periodic RPost had an even more pronounced effect. In an echocardiographic study, which was performed 28 days after coronary artery occlusion, it was demonstrated that RPost improves the pumping function of the heart, and a more pronounced effect on the parameters of myocardial contractility is repeated RPost [31]. In addition, it was found that multiple RPost improved the survival of animals for 84 days after the experimental infarction. Therefore, remote postconditioning can prevent postinfarction heart remodeling.

### 2.5. Cardiac Contractility and RPost

The first publication on the inotropic effect of RPost appeared in 2009 [16]. Studies were performed on rats with coronary artery occlusion (30 min) and reperfusion (2 h). Remote postconditioning was induced using three cycles of occlusion (10 s) and reperfusion (10 s) of the circumflex branch. It turned out that RPost promotes the enhancement of the rate of left ventricular contraction in the reperfusion period. In 2014, Han *et al.* [30] published the results of studies performed on mice with coronary occlusion (30 min) and reperfusion (1 h, 2 h, and 3 h). Remote postconditioning was reproduced using three cycles of ischemia (5 min) and reperfusion (5 min) of the left hind limb immediately after restoration of coronary perfusion. It turned out that RPost promotes an increase in the LV ejection fraction (LV EF) in the reperfusion period [30]. In 2015 J. Xu *et al.* [32] published the results of their experiments on rats ventricular fibrillation (VF). They do not describe the technical details of VF modeling. Remote postconditioning was induced using 4 cycles of ischemia (5 min) and reperfusion (5 min) 5 min after the restoration of normal heart rhythm. It was shown that RPost leads to an increase in cardiac output and an increase in LV EF. The same effect had remote preconditioning. The authors have concluded that remote preconditioning and RPost have a positive effect on cardiac contractility in experimental sudden cardiac death [32].

Thus, the presented data indicates that RPost may be an effective approach for the prevention of reperfusion contractile dysfunction of the heart.

### 2.6. Ventricular Arrhythmias and RPost

In 2013, physiologists published the results of the study on rats with coronary occlusion (45 min) and reperfusion (3 h) [13]. Remote postconditioning was performed using three cycles of ischemia (5 min) and reperfusion (5 min) of the hind limb by occlusion and reperfusion of the femoral artery. The authors showed that DP significantly reduced the incidence of reperfusion ventricular arrhythmias. According to our data [33], after a 45-minute coronary occlusion, single ventricular extrasystoles occur only in individual rats. In most animals, ventricular arrhythmias are absent; therefore, it is not possible to evaluate the effect of any effects on reperfusion arrhythmias. How this was achieved by the Chinese physiologists remains a mystery to us. The results of Zhu's studies coincide with our data [23], they could not detect the antiarrhythmic effect of RPost after a 30-minute coronary artery occlusion.

Thus, the question of whether RPost has an antiarrhythmic effect during ischemia-reperfusion of the heart remains open.

## 2.7. Anti-inflammatory Effect of RPost

It is well known that neutrophil invasion plays an important role in the pathogenesis of cardiac reperfusion injury [34]. In 2006, Li *et al.* [14] in a study on rabbits with coronary artery occlusion (30 min) and reperfusion (3 h), they found out how RPost affects the accumulation of neutrophils in myocardium. This indicator was assessed by the activity of myeloperoxidase, a neutrophil marker enzyme in the area at risk. It turned out that RPost reduces myeloperoxidase activity in the area at risk by more than 50%. In 2009, Fang *et al.* [16] in the experiments on rats confirmed the ability of RPost to reduce neutrophil accumulation in the area at risk. In 2009, Wei *et al.* [31] demonstrated that RPost contributes to the reduction of accumulation of macrophages and neutrophils in the myocardium on the 4th day of reperfusion.

It could be assumed that this RPost effect is a consequence of a decrease in the production of pro-inflammatory cytokines. However, Tang *et al.* [18] demonstrated that RPost did not affect the concentration of interleukin-10 (IL-10) and tumor necrosis factor-α (TNF-α) in rat serum. Another group of investigators failed to detect a decrease in the level of TNF-α and IL-1β in the reperfusion zone [31]. They were measured 4 days after the restoration of coronary perfusion. In addition, they evaluated the level of chemokine macrophage chemoattractant protein-1 (MCP-1) in the reperfusion zone, which is known to cause leukocyte migration to the focus of inflammation [35]. It was established a 6-fold decrease in the this cytokine level in the area at risk. Of course, this effect contributes to limiting the accumulation of neutrophils in the area at risk.

It is possible that the decrease in the production of pro-inflammatory cytokines is related to the protective effect of RPost in the long-term period after the restoration of coronary reperfusion. In 2013, Zhang *et al.* [24] published the results of their experiments on rats with coronary occlusion (30 min) and reperfusion (2 h). Remote postconditioning was induced using single I/R of both hind limbs. It was found that RPost contributes to a decrease in the of serum pro-inflammatory cytokines (TNF-α, IL-1, IL-6) level. The level of these cytokines in the myocardium after RPost also decreased both in the area at risk and in non-ischemic myocardium [24]. It remains a mystery as to why, with such positive dynamics of cytokines, the authors have failed to identify the infarct-limiting effect of RPost.

The presented data suggests that the restriction of leukocyte invasion may be directly related to the cardioprotective effect of RPost. Reducing the level of pro-inflammatory cytokines and the chemokine MCP-1 can promote a limitation of leukocyte infiltration into the reperfusion area.

## 2.8. Effect of RPost on Lipid Peroxidation

The effect of RPost on the level of malonic dialdehyde (MDA) in the blood plasma of rabbits with coronary occlusion and reperfusion [14] was studied. It turned out that the RPost leads to a decrease in the MDA level. This data suggests that RPost suppresses lipid peroxidation. Similar data was obtained by Fang *et al.* [16]. In 2011, Tang *et al.* [19] published the results of their experiments on rabbits with coronary occlusion (30 min) and reperfusion (3 h). They

performed RPost using occlusion (5 min) and reperfusion (5 min) of the pulmonary artery. It was found that RPost contributes to a decrease in the plasma MDA level. Similar data is reported by the same authors in their later publication [36]. In 2014, Wang *et al.* [37] reported on the results of clinical observations of patients with STEMI. They carried out RPost using three cycles of ischemia (5 min) and reperfusion (5 min) of the lower limb. It was found that the RPost did not affect the infarct size, which was determined using serial detection of CK-MB in blood plasma. However, RPost contributed to a decrease in the plasma MDA level.

These facts suggest that RPost provides a reduction in the intensity of lipid peroxidation. It remains a mystery in which organ this "antioxidant" effect is realized, because none of these studies have determined the myocardial MDA.

## 3. CLINICAL DATA ON THE CARDIOPROTECTIVE EFFECT OF RPost

In 2013, Zhong *et al.* [38] published the results of the clinical study on the evaluation of the effectiveness of RPost in children (average age 3.5 years), where cardiopulmonary bypass and cardiac surgery for congenital heart defects was performed. Postconditioning was performed using three cycles of ischemia (5 min) and reperfusion (5 min) of the lower limb. The control group included 35 children, the RPost group - 34 patients. Myocardial necrosis was assessed by serial determination of serum troponin I, CK-MB level before and after the operation (6 h, 12 h, 24 h). The authors found that the RPost caused a significant decrease in the level of markers of cardiomyocyte necrosis. Postconditioning did not affect the level of proinflammatory cytokines in serum. Mean arterial pressure in children with RPost was higher than in controls. The average time spent in the intensive care unit and in the hospital was shorter in children with RPost. The authors concluded that RPost reliably reduced the heart damage during cardiac surgery in children with heart defects and improved the clinical picture of the postoperative period.

In the course of the clinical study of patients with coronary artery bypass grafting (CABG), RPost was shown to reduce necrosis of cardiomyocytes [39], but does not affect the course of the postoperative period [40].

Thus, remote postconditioning prevents reperfusion injury of cardiomyocytes in children and adults, but RPost does not improve the clinical course of the postoperative period in patients with CABG. In children, RPost improves the postoperative period. What is the reason for this difference between children and adults is unclear. It is possible that with RPost in children more humoral factors enter the bloodstream, and therefore it increases the heart's tolerance to ischemic and reperfusion injuries.

In 2013, patients with stable angina and acute coronary syndrome [41] were included in the study. All patients underwent PCI. Myocardial damage was assessed by was the 24-h troponin I peak. The peak troponin in the RIP patients was 0.476 *vs.* 0.478 ng/mL in the control group.

In 2013, Crimi *et al.* [42] reported on the results of their clinical study of patients with STEMI. Immediately after the recanalization of the infarction-related coronary artery,

RPost was carried out using three cycles of ischemia (5 min) and reperfusion (5 min) of the lower limb: 48 patients were included in the RPost group; 48 patients were included in the control group for percutaneous coronary intervention (PCI) only. The time interval between the occurrence of an anginal attack and PCI was on average 3 h. The infarct size was evaluated using the serial determination of serum CK-MB. In addition, a reduction in the ST segment by 50% and 70% at 60 min after PCI was evaluated. It was found RPost reduces CK-MB levels. Patients with RPost had a faster ST segment resolution compared with the control group. In terms of the rate of adverse events (death, stroke, myocardial infarction, CABG, repeated PCI) during the year, the groups did not differ from each other. Cardiologists concluded that RPost has a positive effect on the course of STEMI in the reperfusion period, but it had no effect on the long-term prognosis of the disease [42]. In 2014, F. Prunier *et al.* [43] confirmed the anti-necrotic effect of RPost in patients with STEMI and PCI. In other matters, there is a publication whose author failed to confirm the anti-necrotic effect of RPost in STEMI and PCI patients [37]. In a study performed on patients with STEMI (n = 83), remote postconditioning was induced by four 5-min cycles of arm cuff inflation/deflation [44]. Infarct size was measured by MRI on days 3 to 6 after admission. RPost reduced infarct size by 27% in comparison with control group (p = 0.009). Troponin T level was lower RPost group (p = 0.037). It was found that RPost significantly improved the myocardial salvage index (p = 0.03). In the prospective multicenter trial, 93 patients with STEMI were included which were randomized to RPost or sham procedure [45]. Remote postconditioning was performed by 5-minute cycles of inflation and deflation of a cuff around the left thigh. Infarct size and area at risk were determined by MRI at day 4 to 7. Significant difference in the infarct size and myocardial salvage index between the RPost and control group was not found. Troponin T peak did not differ significantly between the groups. Authors concluded that RPost did not protect against reperfusion injury.

Thus, three publications indicate that RPost has an infarct-reducing effect during PCI in STEMI patients. The authors of the other two articles could not detect the cardioprotective effect of RPost in STEMI and PCI.

In 2017, the results of a study on the cardiac surgery patients (n = 1280) were published [46]. 644 patients were included in the ischemic preconditioning + RPost group, and 636 in the control group. The conditioning was induced with a 5-min inflating cuff. It turned out that conditioning did not affect the rate of adverse events (mortality, myocardial infarction, stroke, and revascularization). Authors have concluded that remote ischemic preconditioning with RPost did not improve long-term adverse events after cardiac surgery.

Thus, the results of RPost clinical trials were not as successful as the results of experimental studies.

## 4. TRIGGER MECHANISM OF CARDIOPROTECTIVE EFFECT OF REMOTE POSTCONDITIONING

### 4.1. The Autonomic Nervous System

It is known that the autonomic nervous system can participate in the cardioprotective effect of remote preconditioning. Thus, in 2009 Jones *et al.* [47], while performing experiments with mice with coronary occlusion (45 min) and reperfusion (24 h), found that the infarct-limiting effect can develop after the dissection of the abdominal wall 15 minutes prior to coronary occlusion. Such a surgical trauma provided a 6-fold decrease in the infarct size/area at risk (IS/AAR) ratio. The infarct-limiting effect of trauma has not manifested after blockade of autonomic nervous ganglions with hexamethonium, after the dissection of the spinal cord, when using local anesthesia with lidocaine, and also after intravenous administration of the blocker of calcitonin gene related peptide (CGRP) receptor. This peptide is released from capsaicin-sensitive sensory nerve fibers and participates in local ischemic preconditioning [48]. This fact allowed Jones *et al.* [47] to suggest that the infarct-reducing effect of trauma could be a consequence of the release of CGRP from the terminals of the afferent fibers. Indeed, it has been found that the subcutaneous injection of capsaicin into the abdominal wall, inducing CGRP release from the afferent fibers, causes a 6-fold decrease in the IS/AAR ratio. This data has allowed the authors to conclude that the nervous system plays an important role in the development of the cardioprotective effect of surgical trauma [47].

In 2012, Wang *et al.* [49] published the results of their experiments in rats with coronary occlusion (30 min) and reperfusion (2 h). Remote postconditioning was simulated with electrical stimulation n. vagus immediately after the removal of the ligature from the coronary artery. It was found that the IS/AAR ratio decreases after the vagal RPost. In addition, stimulation of n. vagus provided a decrease in blood serum levels of markers of necrosis of cardiomyocytes - troponin I, creatine kinase-MB (CK-MB). The authors attribute the infarct-sparing effect of vagal stimulation to the restriction of the production of pro-inflammatory cytokines. Thus, after the vagal RPost in the serum of rats, the level of the following cytokines decreased: tumor necrosis factor-α (TNF-α), interleukin-1 (IL-1), interleukin-6 (IL-6), high mobility group box-1 protein (HMGB-1), intercellular adhesion molecule 1 (ICAM-1). After stimulation of the vagus, a decrease in the level of these cytokines was observed in the intact and ischemic myocardium. The authors have concluded [49] that postconditioning by stimulation of n. vagus provides a decrease in the production of pro-inflammatory cytokines, which leads to the limitation of the size of the infarction. This data was confirmed by Wang *et al.* [50], who found an increase in the HMGB-1 level in the myocardium after I/R. RPost reduced the reperfusion increase in the HMGB-1 level in the myocardium [50].

Relying on this Jones's data, Basalay *et al.* [20]. have tried to evaluate the contribution of the nervous system to the infarct-limiting effect of remote postconditioning. The experiments were carried out on rats with coronary occlusion (30 min) and reperfusion (2 h), in which RPost was modeled by transient occlusion of the femoral artery. It was found that the subcutaneous injection of capsaicin has an infarct-limiting effect only with preventive use. The use of the capsaicin during ischemia or after the coronary perfusion renewal did not affect the IS/AAR ratio. Vagotomy was performed by crossing the right and left n. vagus in the neck. Denervation of the lower limb was performed by crossing the femoral and sciatic nerves. It turned out that denervation

did not affect the infarct-reducing effect of remote postconditioning, if occlusion of the femoral artery was performed at the 10th minute of reperfusion. Vagotomy also did not affect the cardioprotective effect of RPost [20].

Therefore, there are good reasons to assert that the autonomic nervous system does not play an important role in the mechanism of cardioprotective effect of remote postconditioning

Song *et al*. [51] have arrived at an entirely opposite conclusion, who reproduced RPost by surgical trauma, which was reproduced by a cut of the abdominal wall, which was performed at the time of reperfusion after a 45 min coronary occlusion. Surgical trauma contributed to a decrease in the ratio of IS/AAR ratio. Ganglion blocker hexamethonium or antagonist of β-adrenergic receptors propranolol was administered before ischemia [51]. Hexamethonium eliminated the infarct-limiting effect of RPost, and did not affect the ratio of IS/AAR ratio alone. Propranolol eliminated the infarct-limiting effect of RPost, and alone did not affect the IS/AAR ratio. The latter result may be surprising, since the ability of propranolol to limit the infarct size is well known [52, 53]. The authors have concluded that the autonomic nervous system plays an important role in RPost [51].

Thus, at the present time, there is no complete clarity on the question of the role of the autonomic nervous system in RPost.

## 4.2. Humoral Factors Peptide

According to Serejo *et al*. [54], the infarct-sparing effect of remote preconditioning is a consequence of the appearance in the blood of experimental animals of a hydrophobic substance of peptide nature with a molecular weight of about 3.5 kDa. This substance has lost its activity when storing perfusate at room temperature for 24 h or when heated to 70°C [54]. In 2011, Norwegian biochemists attempted to identify a humoral factor which would mediate the cardioprotective effect of RPost [55]. Isolated rat hearts were exposed to 3 sessions of global ischemia (5 min) and reperfusion (5 min). During this pre-coding period, about 150 ml of coronary effluent from the heart were collected and used to treat other preparations of the isolated heart. Other isolated "recipient hearts" were exposed to regional ischemia (30 min) and reperfusion (2 h). The recipients' hearts were perfused for 10 minutes with the collected coronary effluent before 30 minutes of ischemia (pre-coding group) or 10 minutes during reperfusion (postconditioning group). Both impacts provided a 2.5-fold decrease in the IS/AAR ratio. The authors attempted to identify a substance which would mediate the cardioprotective effect of preconditioning and postconditioning. It turned out that this is a hydrophobic substance with a molecular mass of 30 kDa [55]. The authors suggest that the substance is a polypeptide which differs from the known opioid peptides and bradykinin, which are hydrophilic substances substantially inferior to the humoral factor by molecular weight. Several studies have shown that RPost has a neuroprotective effect [56-60]. Therefore, there is the reason to assert that the distinctive feature of the humoral factor is the ability to penetrate the blood-brain barrier. In this connection, it should be noted that this barrier easily overcomes hydrophobic compounds [61]. This data

indirectly confirms the hydrophobic nature of the humoral factor.

## 4.3. Adenosine

In 2005, in experiments on rats Kerendi *et al*. [3] could show that the infarct-reducing effect of RPost, which was reproduced by transient ischemia of the kidneys, is not manifested in the blockade of all types of adenosine receptors by SPT (8-sulfophenyl theophylline). The authors have concluded that the humoral factor which would mediate the cardioprotective effect of RPost is adenosine, released from the kidneys into the bloodstream. In our opinion, this assertion is controversial, since the authors did not take into account the peculiarities of adenosine metabolism: it is quickly captured by red blood cells, so the time of its "half-life" in the blood does not exceed 10 s [62]. Most likely, adenosine is a mediator between a humoral factor and a cardiomyocytes.

## 4.4. Nitric Oxide

An important role in the mechanism of the protective action of DPost is played by the radical of nitric oxide (NO•). Thus, in experiments on rabbits Tang *et al*. [19] have shown that the infarct-limiting effect of RPost is not manifested in the conditions of blockade of NO-synthase with the L-NG-nitro-L-arginine methylester. However, considering the fact that the half-life of NO in biological tissues is 5.6 s [63], it seems unlikely that this compound is the humoral factor that provides cardioprotection. Apparently, NO is the substance that only mediates the infarct-reducing effect of the humoral factor at the heart level. Indeed, remote postconditioning increased the expression of endothelial NO-synthase by cardiomyocytes [36]. This fact suggests that NO plays rather the role of a mediator, than a trigger.

Thus, the most likely RPost trigger is a high molecular weight peptide(s). Nitric oxide and adenosine serve as intermediaries between the peptide and intracellular structures.

## 5. SIGNALING MECHANISM OF RPost

It is known that in the cardioprotective effect of ischemic preconditioning and postconditioning of the following enzymes play a key role: protein kinase C (PKC), kinase MEK1/2 → ERK1/2, kinases PI3 → Akt, STAT3 [4, 5, 8, 64, 65], MEK1/2 is mitogen-activated protein kinase kinase, ERK is extracellular signal-regulated kinase, PI3K is phosphatidylinositol-3-kinase, JAK is Janus kinase, STAT is signal transducer and activator of transcription, Akt is antiapoptotic kinase. Given the similarity of ischemic preconditioning, postconditioning and RPost, there was the reason to believe that these kinases could be involved in RPost. There are two main signaling pathways that increase the cardiac tolerance to the I/R: RISK (Reperfusion-Induced Salvage Kinase) и SAFE (Survivor Activating Factor Enhancement) [8, 66]. MEK1/2, ERK1/2, kinases PI3, Akt are involved in RISK. JAK→ STAT3 are involved in SAFE (Fig. **2**).

## 5.1. Protein Kinase C

In 2013, Gao *et al*. [13] published the results of their experiments in rats with coronary occlusion (45 min) and reperfusion (3 h). Remote postconditioning was induced by
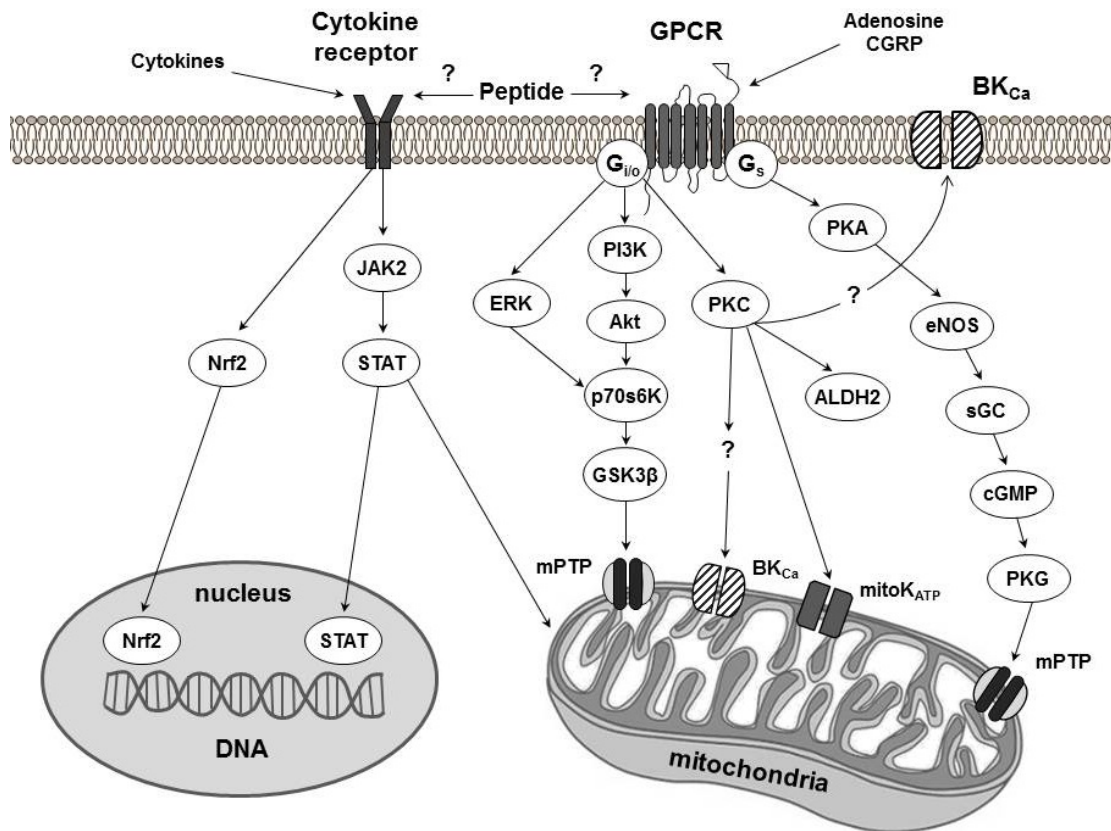
**Fig. (2).** The hypothetical signaling mechanism of infarct reducing effect of remote postconditioning. GPCR, G-protein coupled receptor; CGRP, calcitonin gene related peptide; $G_s/G_{i/q}$, stimulatory/inhibitory G protein; PKA, protein kinase A; PKC, protein kinase C; PI3K, phosphatidylinositol-3-kinase; ERK1/2, extracellular signal-regulated kinase; JAK, Janus kinase; Akt, anti-apoptotic kinase; STAT, signal transducer and activator of transcription; ALDH2, aldehyde dehydrogenase-2; Nrf2, Nuclear factor (erythroid-derived 2)-like 2; $BK_{Ca}$ channel, $Ca^{2+}$ dependent big conductance $K^+$ channel; eNOS, endothelial NO-synthase; GC, guanylyl cyclase; cGMP, cyclic GMP, PKG, protein kinase G; mPTP, mitochondrial permeability transition pore; p70S6K, 70-kDA ribosomal protein s6 kinase; $mitoK_{ATP}$, mitochondrial ATP-sensitive $K^+$-channel; GSK3β, glycogen synthase kinase 3 β.

three cycles of occlusion (5 min) and reperfusion (5 min) of the right femoral artery. Postconditioning was started 15 minutes after the coronary occlusion. It was found out that RPost 2-fold reduces the IS/AAR ratio. The LDH level in the blood plasma after RPost decreased 2-fold. The PKC chelerythrine completely eliminated the infarct-limiting RPost. The participation of PKC in the infarct-reducing effect of RPost, which was caused by surgical trauma, was shown [51].

### 5.2. PI3 Kinase/Akt Kinase

As we noted above, Breivik *et al*. [55] found that after ischemic preconditioning in the coronary effluent, a biologically active peptide appears that can mimic the phenomenon of RPost in experiments on another isolated perfused heart. It has turned out that the inhibitor of PI3 kinase wortmannin or the inhibitor of Akt-kinase SH-6 completely eliminates the infarct-limiting effect of the coronary effluent containing the peptide. In another study, it has been shown that RPost promotes an increase in the amount of Akt kinase in cardiomyocytes [36]. It has been found that RPost causes an increase in the amount of phosphorylated Akt kinase in the myocardium [21]. For most enzymes, phosphorylation indicates an increase in enzyme activity, so the appearance of phosphory-

lated Akt kinase indicates the stimulation of the Akt. Phosphorylation of Akt kinase and activation of PI3 kinase in response to RPost was confirmed in later studies [50, 67].

### 5.3. ERK1/2

Wang *et al*. have found that ischemia-reperfusion leads to an increase in the amount of phosphorylated ERK1/2 [50]. RPost did not cause an additional increase in the amount of phosphorylated ERK1/2.

### 5.4. JAK

JAK kinases belong to cytosolic tyrosine kinases that are specifically associated with cytokine receptors and growth factors: tumor necrosis factor α (TNF-α), leptin, erythropoietin, interleukin 6 (IL-6), interferon-γ, fibroblast growth factor-2 and other cytokines and growth factors [68, 69]. After the interaction with the activated cytokine receptor, autophosphorylation of JAK occurs, after which it phosphorylates STAT [69, 70]. It has been found that inhibition of JAK led to the disappearance of the infarct-limiting effect of Dost [67].

Thus, the results of the presented studies indicate that PKC, JAK, PI3K, Akt are involved in the signaling mecha-

nism of RPost. The contribution to remote postconditioning of protein kinase G, ERK1/2, GSK3β, Src and a number of other kinases participating in ischemic preconditioning remains unclear [64, 71].

## 6. END EFFECTOR OF RPost

### 6.1. Aldehyde Dehydrogenase-2

In 2008, C.H. Chen *et al.* [72] found that PKC-ε phosphorylated mitochondrial aldehyde dehydrogenase-2 (ALDH2). Since PKC-ε plays an important role in cardioprotection, the authors suggested that ALDH2 also contributes to an increase in cardiac resistance to I/R. The infarct-limiting effect of ALDH2 is related to the ability of this enzyme to cleave the cytotoxic aldehydes, which is formed as a result of lipid peroxidation. Phosphorylation of ALDH2 leads to an activation of the enzyme. Immunoprecipitation has shown that both ALDH2 and PKC-ε are precipitated together in the mitochondrial fraction. The authors synthesized the low-molecular activator ALDH2 alda-2. Its introduction to rats contributed to a decrease in the IS/AAR ratio by 60%. This data suggests that ALDH2 plays an important role in regulating heart resistance to the I/R. In experiments on rats with coronary occlusion (45 min) and reperfusion (3 h), it was shown that RPost not only limits the size of the infarct, but also enhances the expression of ALDH2.

### 6.2. STAT

STAT is a well-known target for JAK [70]. It has been shown that RPost leads to phosphorylation of STAT3 [67]. The JAK AG490 inhibitor not only eliminated the infarct-limiting effect of RPost, but also eliminated the phosphorylation of STAT3 [67]. Some authors have not been able to detect STAT phosphorylation after RPost [73], so more research is needed to evaluate the role of STAT in RPost.

It is well known that STAT is a transcription factor. Consequently, it takes time to realize its genomic effects. Whether it can quickly change the transcription to increase the tolerance of the heart to the reperfusion is unknown. Most likely, in the protective action of STAT3, its non-genomic effects are involved [74]. It is believed that STAT3 phosphorylates not only JAK but also other kinases, for example, ERK and PKCε [74, 75]. Thus, it has been shown that phosphorylated STAT3 can penetrate into the mitochondria, where it regulates the electron transport chain, increases the production of ATP, reduces the formation of reactive oxygen species by mitochondria and interacts with the MPT pore (mitochondrial permeability transition pore), which regulates apoptosis [74].

### 6.3. Nrf2

It was found that RPost causes translocation of the transcription factor Nrf2 (Nuclear factor (erythroid-derived 2)-like 2) into the nucleus of the cardiomyocyte [67]. It is known Nrf2 expression is regulated by stromal cell-derived factor-1 alpha (SDF-1α) and interleukin-6 (IL-6) were identified as the upstream modulators of Nrf2 [76]. Nrf2 is known to regulate the expression of the antioxidant cytoprotective proteins (catalase, glutathione peroxidase, superoxide dismutase, hemoxygenase-1) and protect the cell from oxidative stress [77-79], that is, Nrf2, like STAT, provides survival of the cell under adverse conditions. As in the case of STAT, the real contribution of Nrf2 to the cardioprotective effect of RPost remains unclear.

### 6.4. BK$_{Ca}$ Channel

Calcium-dependent $K^+$ channel of large conduction (BK channel from big conductance $K^+$ channel). Sometimes this channel is called "large conductance" or "high conductance". This name is based on the electrophysiological properties of the channel. Among the synonyms should be "Maxi-$K^+$ channel" and "Slo channel". Thus, the abbreviation "hslo" denotes a cloned $Ca^{2+}$-dependent human $K^+$ channel. In the middle of the 1980s, the existence of the $Ca^{2+}$-dependent $K^+$ current of large conductance was an already widely accepted fact [80-82], but the researchers at that time had neither activators nor blockers of the BK$_{Ca}$ channel for their studies. The situation changed in 1992, when iberiotoxin, a peptide consisting of 37 amino acid residues, was isolated from the Buthus tamulus scorpion poison [83]. It is now generally accepted that iberiotoxin is a selective inhibitor of the BK$_{Ca}$ channel.

In 2013, Q. Gao *et al.* [13] published the results of their experiments in rats with coronary occlusion (45 min) and reperfusion (3 h). Remote postconditioning was reproduced with three cycles of femoral artery occlusion (5 min) and reperfusion (5 min). Remote postconditioning exhibited an infarct-limiting effect. This effect was not manifested after the blockade of BK$_{Ca}$ channel by iberiotoxin, which indicates the participation of this channel in postconditioning.

Therefore, today the main contender for the role of the final effector is the BK$_{Ca}$-channel. As for the role of other mitochondrial structures, which play an important role in preconditioning [71], such as the mitochondrial ATP-sensitive K channel, the MPT pore, the question remains open.

## CONCLUSION

Thus, the presented data indicate that the hypothetical trigger for remote postconditioning may be a high molecular weight hydrophobic peptide(s). Investigators have hypothesized that nitric oxide and adenosine serve as intermediaries between these peptides and intracellular structures. The role of the autonomic nervous system in RPost requires further study. There is mounting evidence that the following kinases play an important role in the remote postconditioning signaling mechanism: protein kinase C, PI3-kinase, Akt kinase, and JAK. The role of the hypothetical end effector for RPost has been postulated to be: aldehyde dehydrogenase-2, the transcription factors STAT, Nrf2, and also the BK$_{Ca}$-channel.

## CONSENT FOR PUBLICATION

Not applicable.

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## CONFLICT OF INTEREST

The author declares no conflict of interest, financial or otherwise.

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