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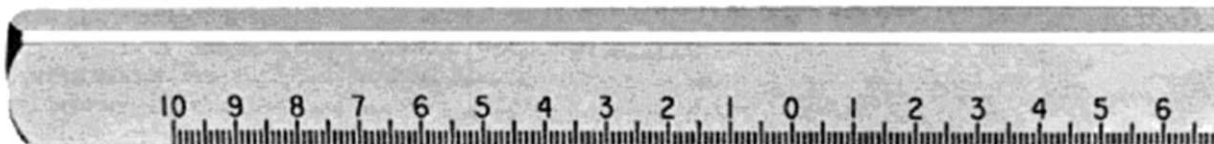
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Single Preconditioning Stimulus Becomes Stronger When Combined with ACE Inhibitor Spirapril in the Rat Model of Myocardial Ischemia/Rep erfus ion

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INTRODUCTION

The effects of angiotensin-converting enzyme (ACE) inhibitors on cardiac remodeling associated with congestive heart failure (CHF) following myocardial infarction (MI) have been the subject of considerable research. Pfeffer et al. showed that chronic treatment with captopril reduced left ventricular enlargement [1] and also prolonged survival [2] after MI in the rat model. Other ACE inhibitors (e.g. enalapril) also improved survival of animals with CHF after MI [3, 4]. Besides, it has been found that spirapril and zofenopril equally attenuated both ventricular enlargement and cardiac hypertrophy in rats with CHF after Ml provided that treatment was started in (he acute phase of MI [5]. Although the beneficial effects of ACE inhibitors in CHF after MI are now well recognized, the potential cardioprotective status of these drugs in the settings of acute myocardial ischemia remains questionable.

A number of studies indicate that ACE inhibitors may be effective in reducing (he extent of ischemic injury alone as well as ischemic injury associated with reperfusion ones.

For example, Ertl et al. [6] showed that intravenously administered captopril is potent in reducing infarct si/e in a canine model. On the other hand, some investigations produced negative results regarding the salvageable effects of ACE inhibitors on the ischemically compromised tissue. Black et al. [7] failed to demonstrate any cardioprotective action of both captopril and ramiprilal on myocardial infarct size under conditions of ischemia alone or ischemia with reperfusion in the dog. The controversies observed are probably due to the differences in animal species used, a technique employed (in situ, in vitro), a method of drug administration (intravenous, intracoronary), its dose, and duration of action (single bolus or prolonged infusion).

The mechanisms of the putative salutary actions of ACE inhibitors on ischemic myocardium are also remain to be elucidated. One possibility includes the improvement of collateral blood flow to the jeopardized tissue thus saving viable edges of the zone of necrosis. Another mode of action suggests that ACE inhibitors are able to decrease preload and afterload conditions by virtue of reducing the systemic reflex vasoconstriction. The latter may result in optimizing the balance between increased myocardial oxygen demand and decreased myocardial oxygen supply [6j. In the light of recent data on the existence of local intracardiac renin-angiotensin-aldosteron system (RAAS), this pathway can be significantly modified by ACE inhibitors administration. Further, ACE inhibitors might exert beneficial effect on ischemia/reperfusion induced injury by the accumulation of bradykinin in the cardiac interstitium [8]. In the ischemic heart the enhanced generation and release of kinins seem to have cardioprotective actions. In isolated rat hearts with ischemia-reperfusion injuries, perfusion with bradykinin reduces the duration and incid ence of ventricular fibrillation, improves cardiodynamics, reduces release of cytosolic enzymes, and preserves energy -rich phosphates and glycogen stores [9]. In anesthetized animals, intracoronary infusion of bradykinin is followed by comparable beneficia 1 changes and limits infarct size [10].

Bradykinin is also known as a potential trigger of ischemic preconditioning phenomenon. Along with early reperfusion, ischemic preconditioning (IPC) is generally known to be a most effective means of ischemic heart damage prevention [11]. The IPC phenomenon was introduced in 1986 by Murry et al. f 12] who found that several brief bouts of ischemia prior to a prolonged ischemic insult resulted in a robust decrease in infarct size. Generally, IPC is considered to be a substantial increase in resistance of an organ to prolonged ischemia after one or few brief ischemic challenges [13]. It should be noted that a variety of endogenous substances including adenosine |15], bradykinin [16], acetylcholine [17], and norepinephrine [18] are implicated in the mechanism of IPC. The evidence for bradykinin involvement in the mechanism of IPC was presented by Pan et al. [19] who showed an increased interstitial bradykinin level in the feline myocardium stressed by a brief bout of regional ischemia The trigger role of bradykinin in the IPC was further supported by the observation that the selective B2 -receptors blocker, ikatibant (HOE 140) is able to abolish infarct-limiting effect of IPC in the rabbits [20].

Protective effects of ACE inhibitors in myocardial ischemic and reperfusion injury have been attributed to their non-ACE-inhibitory actions. Sulfhydryl (SH) containing ACE inhibitors, particularly captopril, have been shown to have antioxidanl activity [21]. Captopril has also been implicated in the scavenging of the hydrogen peroxide radicals [22].

The aim of this study was to examine the possible infarct-limiting action of ACE inhibitor spirapril in the rat model of myocardial ischemia/reperfusion. Another goal was to investigate how, if any, and to what extent the infarct-limiting potency of the IPC can be modified by spirapril administration. It is possible that ACE inhibition by means of elevating the tissue bradykinin level can make the preconditioning stimulus stronger and, thus, to confer an additional protection to the ischemic heart.

METHODS

Surgical Procedures

All experiments were performed in accordance with the "Guide for the Care and Use of Laboratory Animals" (publication No. [NIH] 85-23) and were approved by the institutional ethical committee of the Pavlov State Medical University of St. Petersburg. Male Wistar rats, 250-350 g body wt used throughout of the experiments were anesthetized with sodium pentobarbital (60 mg/kg ip). The trachea was intubated through a cervical incision. Mechanical ventilation was achieved with a positive-pressure respirator using room air, with a tidal volume of 4.5 ml, and a rate of- 50 breaths/min. The left carotid artery and femoral vein were cannulaled for blood pressure monitoring and anesthesia maintenance, respectively.

ECG lead II was monitored for registration of rrhythm disturbances. A rat model of coronary artery occlusion and reperfusion was used as described previously [23]. Briefly, the chest was opened by left thoracotomy through the fourth intercostal space, and the epicardium was exposed via the pericardium incision. A 6-0 polypropylene thread was passed around a prominent branch of the left coronary artery (LCA). The thread then was made into an overhand knot, an occluder, and two more threads were tied to the main knot (releasers). The tips of all of the threads were brought outside the thoracic cavity. Thus the occlusion could be either tightened or loosened by pulling the appropriate threads.
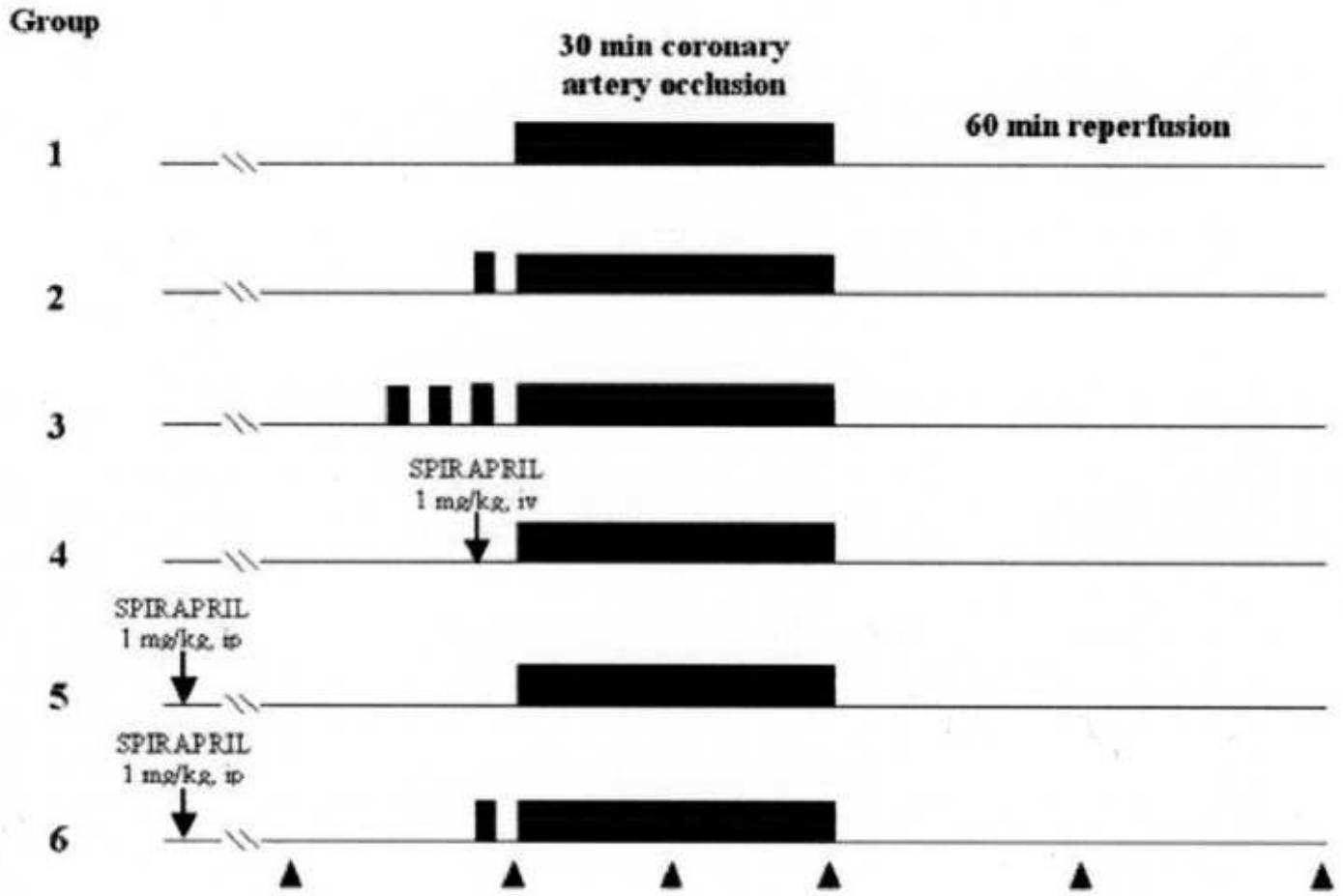
Figure. 1 Study design A . measurements of mean blood pressure and heart rate

Experimental Protocol

The rats were randomized to one of six groups at the beginning of the study (Fig. 1):

1. *Control (nonpreconditioned) group (10 animals).* The LCA was occluded for 30 min followed by 1 h of reperfusion without preconditioning
2. *Preconditioning 1x5'group (6 animals).* Test ischemia was preceded by one cycle of 5 min ischemia/reperfusion.
3. *Preconditioning 3x5 'group (9 animals).* The IPC protocol included three 5 min cycles of ischemia each spaced by 5 min phases of reperfusion. It was followed by a sustained 30 min occlusion and 1 h of reperfusion. To protect LCA against the excessive knot pressure over the repetitive preconditioning occlusions, a thin length of teflon tubing was positioned between the knot and the artery wall.
4. *Control + Spirapril iv group (6 animals).* One dose of spirapril (1 mg/kg) was given intravenously 5 min before the sustained 30 min of coronary occlusion and 1 h of reperfusion.
5. *Control + Spirapril ip group (6 animals).* A dose of spirapril (1 mg/kg) was administered intraperitorEally 1.5-2 h prior to the sustained 30 min of coronary occlusion and 1 h of reperfusion.
6. *Preconditioning 1x5' + Spirapril ip group (6 animals).* A dose of spirapril (1 mg/kg) was administered intraperitoneally 1.52 h before preconditioning. The same regimen was used to precondition the hearts as in the Group 2. Then the coronary artery was occluded for 30 min and reperfused for 1 h.

Measurements

Hemodynamics. Measurements of heart rate and mean arterial pressure were made in all groups at baseline (that is, before opening the chest), immediately before the sustained occlusion, 15 min after occlusion, at the end of the 30-min occlusion. 30 min after reperfusion, and at the end of the experiment.

Infarct Size and Area at Risk. Assessment of myocardial infarct size was done by macroscopic staining with triphenyltetrazolium chloride (TTC). The region of the heart that included necrotic as well as ischemic but still viable tissue was designated as an anatomic area at risk (AR). At the end of the experiments, the LCA was reoccluded followed by the administration of 0,5 ml of 5% Evans blue solution through the left femoral vein for identification of AR that resulted in identification and easy delineation of the area by the absence of blue dye. The rat was then sacrificed by KCI injection while under deep anesthesia, and the heart was excised. The atria and were removed, the ventricular cavities were carefully rinsed with saline, and the hearts were cut into four slices (2-3 mm thick) parallel to the atrioventricular groove. The basal surface of each slice was photographed using digital camera Camedia Olimpus 2020. After that, the slices were immersed in 1% solution of TTC at 37° (pH 7.4) for 15 min. A lack of TTC staining within the AR was considered to be a sign of necrosis in agreement with the previous studies [25]. After immersion in TTC, the slices were photographed again and weighted. The images of the basal surface of the slices were digitized using VideoTest 4.0 (ISTA Ltd.) Software for determination of the AR and AN size. The calculated areas of each slice of every heart were then normalized to slice weight values to be summarized [26]. The relative volume of tissue at risk was expressed as a percentage to the whole heart volume (AR/HV), and the relative volume of the necrotic tissue was further expressed as a percentage to the volume of the tissue at risk (AN/AR). The animals with relative AR volumes below 15% of the heart volumes were excluded from the study.

Arrhythmia. The primary end point of this study was infarct size. However, we also analyzed the electrocardiogram for the occurrence and duration of arrhythmais. Arrhythmia during either ischemia or reperfusion was categorized as ventricular tachyarrhythmia (TA). TA was defined as tachycardia or fibrillation. The distinction between tachycardia and fibrillation was difficult in this model because of numerous intermediate grades in the morphology of TA and a tendency to interconvert from one type to another. Analyses were carried out in accordance with the Lambeth Conventions [24].

Chemicals

Spirapril hydrochloride was a gift from AWD, Germany. Evans blue and tripheniltetrazolium chloride were purchased from ICN Pharmaceuticals Co.

Statistics

Differences between the groups were evaluated using Student's paired samples test and Wilcoxon-Mann-Whitney approach with further PC analysis using software STATISTICA 98, Inc. All data are presented as means $\pm$ SD, and a p value < 0.05 was accepted as indicating a significant difference in mean values.
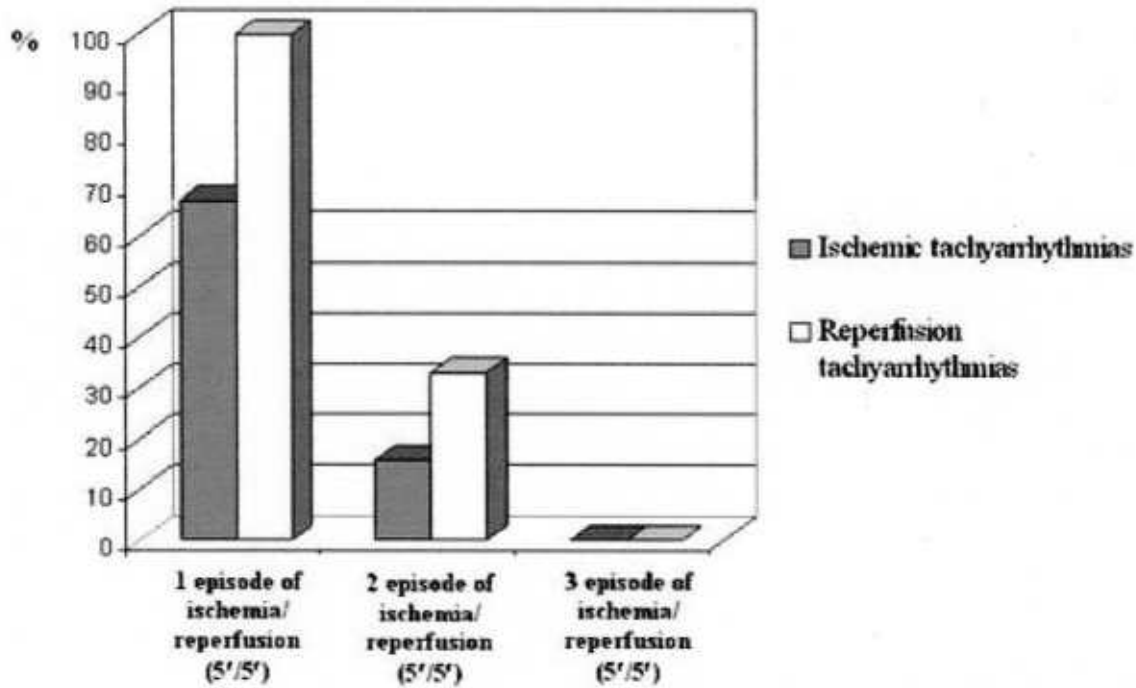
RESULTS

Mortality and exclusions

Totally, 43 animals were entered the study. One rat in the control group died perioperatively due to persistent VF which occurred on the 7th min of ischemia. Two rats in the IPC 1x5' group and three more animals in the IPC 3k5' group died due to persistent VT induced by reperfusion after first 5-min LCA occlusion. One rat (control group) was excluded from the study because of too small AR size (< 15% of total heart volume) as measured by in vivo dye injection. Thus, 37 animals were included in the final analysis: 8 in the control group, 6 in the IPC 1x5' group, 4 in the IPC 3x5' group, 6 in the spirapril iv group, 6 in the spirapril ip group, and 6 in the IPC Ix5'+spirapril ip group.

Table 1 Summary- of lieiiiodynamic variables in experimental a> onps * - p<0.05 in companion with controls.

Group	1	2	3	4	5	6
Heart rate, beats/min						
Baseline	378±12	388±14	396±25	413±15	402±9	398±13
Preocclusion	385±28	405±13	406±18	382±11	408±10	395±15
Ischemia:						
- 15 min	382±15	375±9	386±28	390±23	395±16	401±17
- 30 min	355±11	383±19	373±9	407±18	388±15	372±8
Reperfusion:						
- 30 min	358±13	412±14	398±27	422±11	395±13	371±16
- 60 min	369±22	410±21	387±19	397±18	399±18	370±22
Mean blood pressure, mm Hg						
Baseline	106±12	109±5	115±9	125±18	118±13	115±10
Preocclusion	96±6	98±8	104±10	78±6*	110±12	100±7
Ischemia:						
- 15 min	88±11	72±6	89±7	80±4	75±14	78±9
- 30 min	96±8	84±7	81±15	85±8	92±4	95±7
Reperfusion:						
- 30 min	65±17	66±8	73±7	78±17	79±7	75±10
- 60 min	68±12	65±5	69±13	72±9	68±8	71±11

Hemodynamic data

Hemodynamic data from all of the groups are summarized in Table 1. Baseline heart rates and mean blood pressures were not significantly different among groups with exception of group 4 were intravenous administration of spirapril caused a significant decrease in mean arterial pressure (78±6 mm Hg versus 96±6 mm Hg in the controls, p<0.05), and this effect was evident within a few minutes following the injection of drug.

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Figure 2 Frequency of isclieinic and reperfiision tacliyarrhytinntas diuitts preconditioning protocol The data on anliythmia incidence during first 5 nun ischemia and fast 5 uun reperfiisioii me summarized for group 2 and 3.

Arrhythmias

The first 5-min preconditioning ischemia/reperfusion episode resulted in 100% occurrence of reperfiision tachyarrhythmias, among which 33.3% in group 3 and 33.3% in group 2 were persistent and caused animal death. The second bout of 5 min ischemia followed by 5 min reperfusion caused transient runs of VT or VF in 33.3% cases only, whist after third brief ischemic challenge there were no tachyarrhythmias at all (Fig. 2)

The incidence of ischemic arrhythmias during preconditioning was characterized by a similar pattern. During the first 5 min occlusion arrhythmias occurred in 65% (this value has been obtained by summarizing the data from group 2 and 3), during the second 5 min occlusion (group 3) - in 16%, and during the third 5 min occlusion no arrhythmias occurred.

Table 2 summarizes the data on the occurrence and duration of ischemic arrhythmias. It should be noted that no rrhythm disturbances (i. e. ventricular tachyarrhythmias) were registered within the reperfusion period. During the test ischemia in the control group 50% of animals had one or more paroxysms of ventricular fibrillation (VF), and ventricular tachycardia (VT) episodes occurred in 6 of 8 nonpreconditioned animals. In contrast, no VT or VF occurred in the IPC group during 30 min of ischemia.

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Table 2. Anliythnua* duriu$ 30-nun coronary occlusion ui the groups 1-3

	Controls	IPC (1×5′)	IPC (3×5′)
Number of animals	8	4	6
Ventricular fibrillation			
Frequency, %	50	0	0
Number of episodes, per animal	1,6±0,53		
Total duration, min	1,7±1,24		
Ventricular tachycardia			
Frequency, %	75	25	0
Number of episodes, per animal	2,4±0,22	2	
Total duration, min	0,89±0,023	0,48	

Table 3 Relative volume of the anatomical area at risk and nifarcted area * - $p<0,05$, ** - $p<0,01$ m conipniison with controls.

Group	Risk zone (% of heart volume)	Necrotic zone (% of area at risk volume)
Controls (n=8)	40,4±6,32	56,0±11,32
IPC 1×5′ (n=4)	43,5±8,21	48,9±9,92
IPC 3×5′ (n=6)	42,3±5,74	10,44±2,35**
Spirapril iv (1 mg/kg) 10 min prior MI (n=6)	39,1±7,67	52,0±10,17
Spirapril ip (1 mg/kg) 1,5-2 h prior MI (n=6)	42,1±5,48	45,5±8,70*
Spirapril ip (1 mg/kg) 1,5-2 h prior MI+IPC 1×5′ (n=6)	44,0±6,55	25,6±4,68**
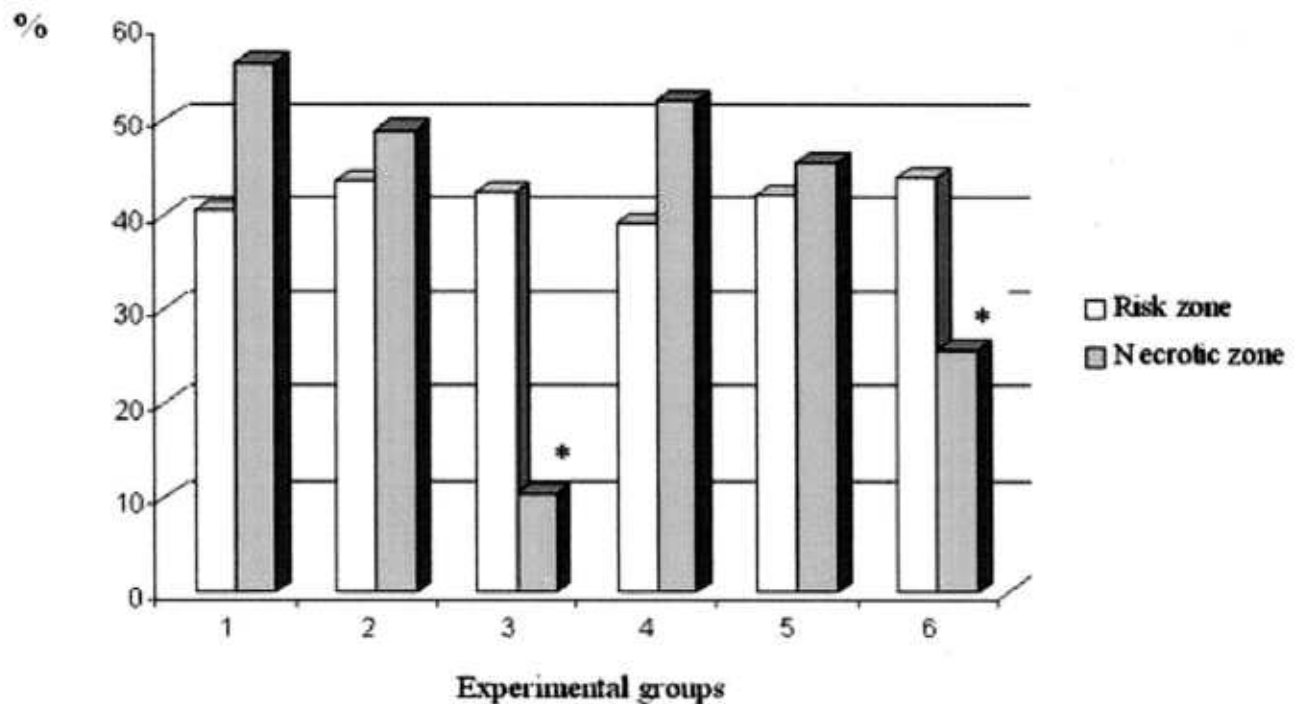
Fig, 3 lufarct size and anatomic area at risk ui the experimental groups * - p<0,05. Area at risk and Area of necrosis

The anatomic AR was comparable among groups and averaged in 41,816,32% (Table 3, Fig. 3). Myocardial infarct size, expressed as AN/AR, averaged 56,0111,32% in the control group. There was no significant difference in myocardial infarct size between control and IPC 1x5 group (56,0111,32% and 48,919,92%, respectively, p=NS), although the latter group displayed a tendency towards lesser infarct size. IPC 3<5' caused a dramatic decrease in infarct size as compared to the controls (10,4412,35 vs. 56,0±11,32%, p=0,000071). Of interest is the necrosis pattern within the ischemic zone: in the control group there was a single confluent necrotic area surrounded with the unaffected tissue whereas the group showed patches of necrosis intermingled with the areas of ischemic albeit viable myocardium.

Intravenous spirapril administration 10 min before sustained ischemia failed to limit infarct size, but in the same dose 1,5-2 hours prior to MI caused statistically significant reduction of infarct area volume (45,5±8,70 vs. 56,0111,32% in control, p=0,0491). When IPC 1x5' was preceded by intraperitoneal injection of spirapril (1 mg/kg) infarct size was reduced up to 25,614,68% (p<0,01).

DISCUSSION

The present study has confirmed many other investigations showing the effectiveness of IPC both against infarction and ischemic and/or reperfusion arrhythmias. We employed a protocols included one or three brief ischemic episodes for the estimation of infarct-limiting IPC potency. Liu and Downey, 1992 [14] found in the rat model that the threshold for infarct-limiting IPC effect is somewhat higher than that for its antiarrhythmic potency in this particular species in contrast to the rabbit and dog. These authors observed an attenuation of ischemic tachyarrhythmias as a consequerce of single 5-min cycle of preconditioning but this protocol was absolutely ineffective in terms of infarct-limitation. A sufficient protection against infarction did occur only if three Smin cycles of ischemia/reperfusion were employed prior to prolonged ischemia. However, Schoemaker et al. [27] have demonstrated in the similar model that a single 15 min ischemia followed by 10 min reperfusion causes a sufficient decrease in myocardial infarct size induced by 60 min coronary occlusion. In our

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experiments we failed to see any statistically proved decrease in infarct size when the hearts were preconditioned by one 5 min cycle of ischemia/reperfusion while three episodes of 5 min ischemia leaded to a robust mfarct-limitation. Hence, the more strong ischemic challenge causes the more significant protection. There were no significant differences in hemodynamics between groups, which provides an additional evidence for local (intracardiac) nature of the protective IPC mechanism. The pattern of antiarrhythmic IPC action showed in the present study is in agreement with Liu and Downey data about equally protective action of a single and multiple coronary artery occlusion(s) on the ischemic arrhythmias [14]. Three 5 min occlusions as well as one were able to completely abolish the occurrence of ventricular fibrillation during prolonged ischemia.

Analysis of the arrhythmia incidence during IPC protocol revealed that the most arrhythmogenic condition producing high incidence of persistent (i. e. lethal) forms of ventricular fibrillation is reperfusion after the first 5 min occlusion. These results are consistent with previous investigations. In particular, it is generally known that in the rat just 5 minute coronary occlusion followed by reperfusion produces the most severe rrhythm disturbances [28]. In the study of Hagar et al. [29] rats underwent 5 minutes of test ischemia followed by reperfusion which was preceded by a series of three 2 min occlusions. The authors observed marked protective effect of such a preconditioning regimen against reperfusion-induced arrythmias. However, for the assessment of infarct-limiting effect of IPC being major in our study the several 5 min occlusions are most commonly used. Therefore, the fist 5 min episode of ischemia supported by the following two similar episodes along with a significant protection against infarction brings a great deal of jeopardy of lethal reperfusion arrhythmias occurrence. These considerations encouraged us to suggest a rationale for using a protocol including he first bout of 2 min ischemia serving as a preconditioning against severe, life-threatening reperfusion arrhythmias following by two 5 min occlusions which afford protection against infarction.

In the recent years, much more attention has been paid to the concept of pharmacological preconditioning, i. e. the possibility to induce ischemic preconditioning in human heart by pharmacological means. In particular, the strategies aimed at lowering the threshold for preconditioning are considered as the effective approach to protecting human heart [30]. For example, the myocardial adenosine level elevating agent acadesine contributed to the manifestation of infarct-limiting action of a single 2 minutes episode of ischemia/reperfusion in the rabbit [31]. The same preconditioning regimen or acadesine administration were ineffective when employed separately. Therefore, acadesine lowered the temporal threshold for preconditioning in the rabbit to below 2 min of ischaemia while in the normal conditions only 5 min ischemia confers a significant protection. In the similar model, Miki et al. [32] observed a significant bradykinin-dependent infarct-limiting effect of a single 2 min ischemia/reperfusion on the background of captopril administration (1 mg/kg). Injection of captopril alone in the same route did not alter myocardial infarct size.

In our studies spirapril at a dose 1 mg/kg given intravenously 10 min before coronary occlusion had no influence on myocardial infarct size. The absence of cardioprotective effect in this route of administration could be explained by a significant hypotensive effect associated with the decline in coronary perfusion pressure. The alternative explanation is that there was not enough time for biotransformation of spirapril into its active metabolite -spiraprilat in the settings of its acute injection. Infarct size was significantly lower when spirapril was administered intraperitoneally 1,5-2 hours prior to infarction, although no hemodynamic shifts were observed in this case. The most prominent infarct-limiting effect was seen when one 5 min occlusion was employed together with intraperitoneal spirapril administration. Potentiation of cardioprotective IPC action noticed in the group that received 1x5' ischemia plus spirapril enables us to suppose some degree of IPC threshold lowering with ACE inhibitors.

In conclusion, the data presented generally confirm the existence of various aspects of cardioprotection afforded by IPC and provide a substantial piece of evidence that ACE inhibitors (particularly, spirapril) may lower the threshold for preconditioning.

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