Inotropic and Chronotropic Effects of Ischemic Postconditioning on the Model of Isolated Heart

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 152, No. 12, pp. 627-630, December, 2011
Original article submitted July 1, 2010

The effects of global ischemia (45 min) and reperfusion (30 min) on left-ventricular developed pressure (LVDP), HR, and end-diastolic pressure were studied on isolated perfused rat heart. The following postconditioning protocols were used: 1) 3 cycles of reperfusion (10 sec) and ischemia (10 sec), total cycle duration 20 sec; 2) 6 cycles reperfusion of reperfusion (10 sec) and ischemia (10 sec), total cycle duration 20 sec; 3) 3 cycles of reperfusion (20 sec) and ischemia (20 sec), total cycle duration 40 sec; 4) 6 cycles of reperfusion (20 sec) and ischemia (20 sec), total cycle duration 40 sec; 5) 3 cycles of reperfusion (30 sec) and ischemia (30 sec), total cycle duration 60 sec. The use of several cycles of a total duration of 20 sec had no effect on LVDP, but reduced EDP throughout the reperfusion period. Postconditioning protocol consisting of three 40-sec cycles promoted LVDP recovery during the reperfusion, but had no effect on EDP and decelerated HP normalization. Six 40-sec cycles had no effect on LVDP and EDP, but impeded HR recovery during the reperfusion period. Postconditioning protocol consisting of three 60-sec cycles promoted LVDP increase during the reperfusion and reduced contracture, but these transient effects were accompanied by decelerated HP normalization.

Key Words: *heart; ischemia; reperfusion, postconditioning*

Ischemic postconditioning phenomenon was discovered in 2002 by a group of American physiologists headed by J. Vinten-Johansen [6]. Experiments were carried out on dogs with 60-min occlusion followed by 3-h reperfusion of the left descending coronary artery (control group). It was demonstrated that three 30-sec reperfusion periods alternating with 30sec coronary occlusion periods made the myocardium more resistant to reperfusion injuries. Further studies showed that this ischemic postconditioning phenomenon (IP) could be reproduced in experiments on isolated perfused heart [1-3]. In this case IP prevents the development of reperfusion contractile dysfunction of the heart [1-4]. However, according to some views IP cannot influence the disturbances in pumping function of the isolated heart during the reperfusion period [5]. There is no common *in vitro* model of IP. Some authors use 3 cycles consisting of alternating 10-sec reperfusion and 10-sec ischemia periods, total cycle duration 20 sec [2]. Others use 6 cycles of reperfusion and ischemia, 20 sec each [4]. Physiologists often use 3 reperfusion-ischemia cycles with total duration of 60 sec [1].

Here we evaluated the inotropic and chronotropic effects of different *in vitro* models of cardiac IP.

MATERIALS AND METHODS

Experiments were carried out on isolated hearts from male Wistar rats weighing 250-300 g narcotized with diethyl ether. After the heart was promptly removed from the thorax and arrested in cold (4°C) Krebs–Henseleit solution, the ascending aorta was cannulated, and retrograde Langendorff perfusion with Krebs–Henseleit solution was performed. Oxygenated perfusion solution (37°C, pH 7.4) contained

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0007-4888/12/1526-0688

(in mM): 120 NaCl, 4.8 KCl, 2.0 $CaCl_2$, 1.2 $MgSO_4$, 1.2 KH_2PO_4, 20.0 $NaHCO_3$, and 10.0 glucose (all reagents were from MP Biomedicals, Irvin). Perfusion pressure was maintained at the level of 52 mm Hg.

For recording of heart contractility parameters, a catheter with latex balloon filled with distilled water was introduced into the left ventricle; the volume of the balloon was sufficient for creating end-diastolic pressure of 10-15 mm Hg. Parameters of pumping function of the heart were measured in isovolumic regimen using an SS13L pressure transducer (Biopac System Inc.) connected to the balloon. Left-ventricular pressure was recorded using an MP35 device for electrophysiological studies (Biopac System Inc.). The data were processed using INSTBSL-W software (Biopac System Inc.). During the experiment, HR (bpm) and left-ventricular developed pressure (LVDP, mm Hg) were recorded. The latter was calculated as the difference between the systolic and diastolic pressure. End-diastolic pressure (EDP, mm Hg) was also measured.

In the control group, the heart after 20-min adaptation to normoxic perfusion was subjected to 45-min global ischemia and 30-min reperfusion.

The following postconditioning protocols were used: 1) 3 cycles of reperfusion (10 sec) and ischemia (10 sec), total cycle duration 20 sec; 2) 6 cycles reperfusion of reperfusion (10 sec) and ischemia (10 sec), total cycle duration 20 sec; 3) 3 cycles of reperfusion (20 sec) and ischemia (20 sec), total cycle duration 40 sec; 4) 6 cycles of reperfusion (20 sec) and ischemia (20 sec), total cycle duration 40 sec; 5) 3 cycles of reperfusion (30 sec) and ischemia (30 sec), total cycle duration 60 sec. Each group comprised 14 hearts. Since the experiments were performed during fall and winter, control experiment (ischemia–reperfusion) and experiment for postconditioning were daily carried out to exclude the effects of seasonal variations in heart tolerance to ischemia-reperfusion.

The results were processed using Mann–Whitney test.

RESULTS

In the control group, LVDP dropped to 13% of the control value in 5 min after 45-min ischemia (Fig. 1). Similar dynamics of LVDP was observed after 3 cycles of reperfusion and ischemia. Five minutes after the start of reperfusion, HR in the control group was 52% below the initial value (Fig. 2). In case of preconditioned heart, HR also decreased by 52%. EDP in the control group on the 5th minute of reperfusion surpassed the initial value by 5.5 times (Fig. 3), while in IP group EDP increased by only 4.5 times. These differences in EDP between the groups were statistically insignificant. Hence, IP consisting of 3 cycles of reperfusion and ischemia (20 sec) prevented the development of reperfusion contracture, but had no significant effect on HR and LVDP.

In the next experimental series we showed that LVDP in the control group on the 5th minute of reperfusion was 12% of the initial value (Fig. 1). In case of heart postconditioned in 6 cycles of reperfusion and ischemia (20 sec), LVDP decreased by 85%. In the control, HR after 5-min reperfusion decreased by 2.2 times in comparison with the initial value, while in IP group this parameter decreased 2-fold (Fig. 2). EDP in the control group increased by 5.5 times, while in hearts subjected to IP this parameter increased by 4.2 times (Fig. 3). These differences in EDP between the groups were statistically significant. These findings

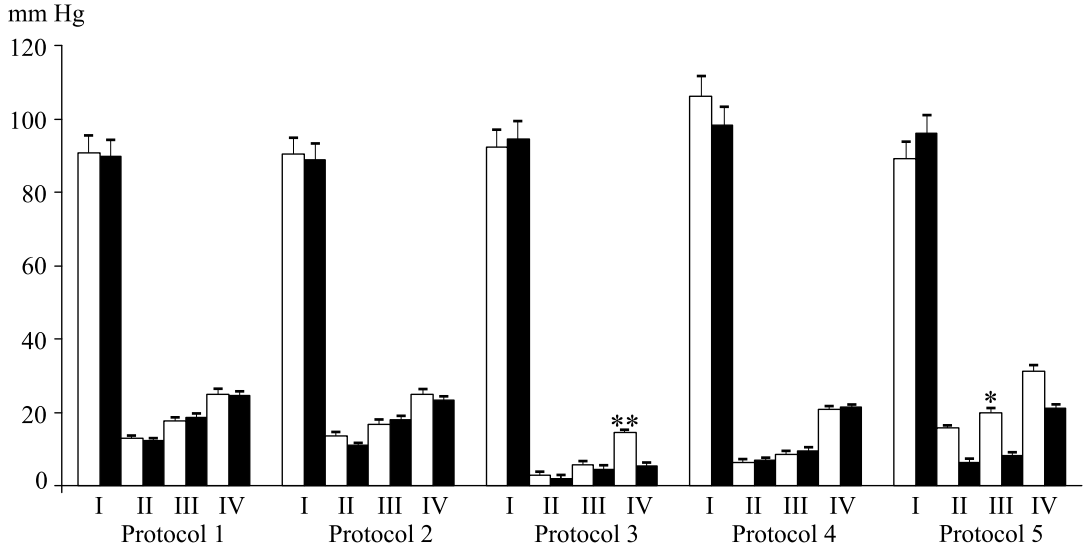

Fig. 1. LVDP after IP. Here and in Figs. 2 and 3: I: 20-min adaptation, II: 5-min reperfusion, III: 15-min reperfusion; IV: 30-min reperfusion. Open bars: IP; dark bars: control. *p<0.05, **p<0.01 in comparison with the control.

suggest that postconditioning consisting of 6 reperfusion ischemia cycles (20 sec) reduced EDP, but had no effect on reperfusion-induced decrease in contraction force and HR.

In the control group, LVDP dropped to 2% of the control value 5 min after the start of reperfusion (Fig. 1). The same value was observed in hearts subjected to 3 postconditioning cycles of 40 sec duration. However, after 30-min reperfusion LVDP was 5.8% of the initial value in the control group and 15.5% in the postconditioning group. These differences between the groups were significant. Pronounced bradycardia was recorded after 5-min reperfusion in both the control and postconditioning groups. After 30 min, partial normalization of the heart rate was seen in the control group while in the postconditioning group pronounced bradycardia persisted (Fig. 2). In both group, practically identical increase in EDP was observed after ischemia–reperfusion (Fig. 3). Hence, IP consisting of three 40-sec cycles increased the force of heart contractions, but had no significant effect on EDP. Unfortunately, postconditioning aggravated reperfusion bradycardia.

Postconditioning consisting of 6 cycles of reperfusion and ischemia (40 sec) had no significant effect on reperfusion values of LVDP and EDP (Fig. 1, 3), but aggravated reperfusion bradycardia (Fig. 2). Hence, IP consisting of six 40-sec cycles produced no positive effect on the pumping function of isolated heart.

After three 60-sec cycles of reperfusion and ischemia, we observed a 2.4-fold increase in LVDP (Fig. 1) and a decrease in EDP (Fig. 3) in comparison with the control on the 15th min of reperfusion. At the same time, postconditioning decelerated HR recovery: HR

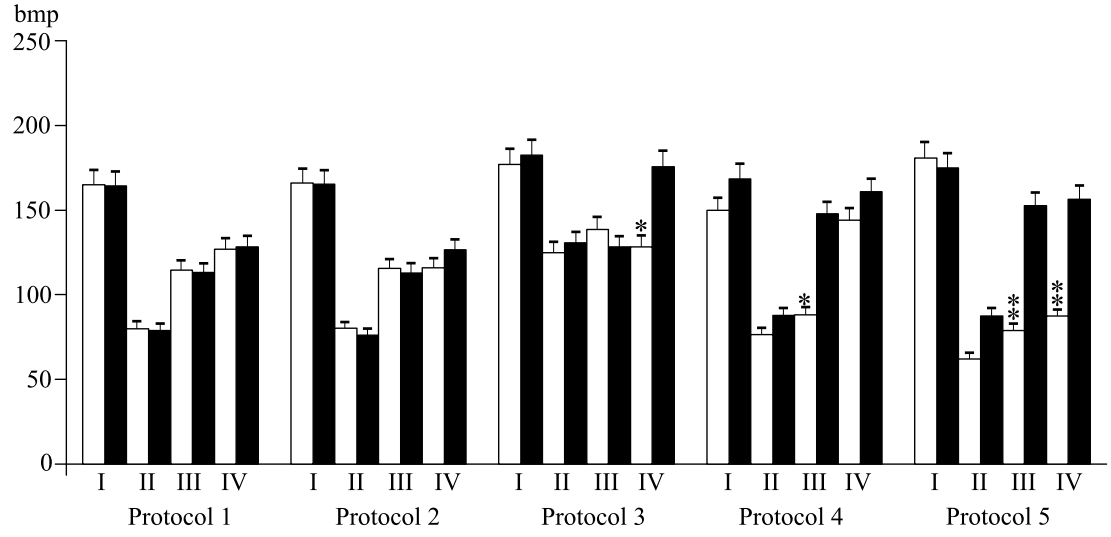

Fig. 2. HR after IP.

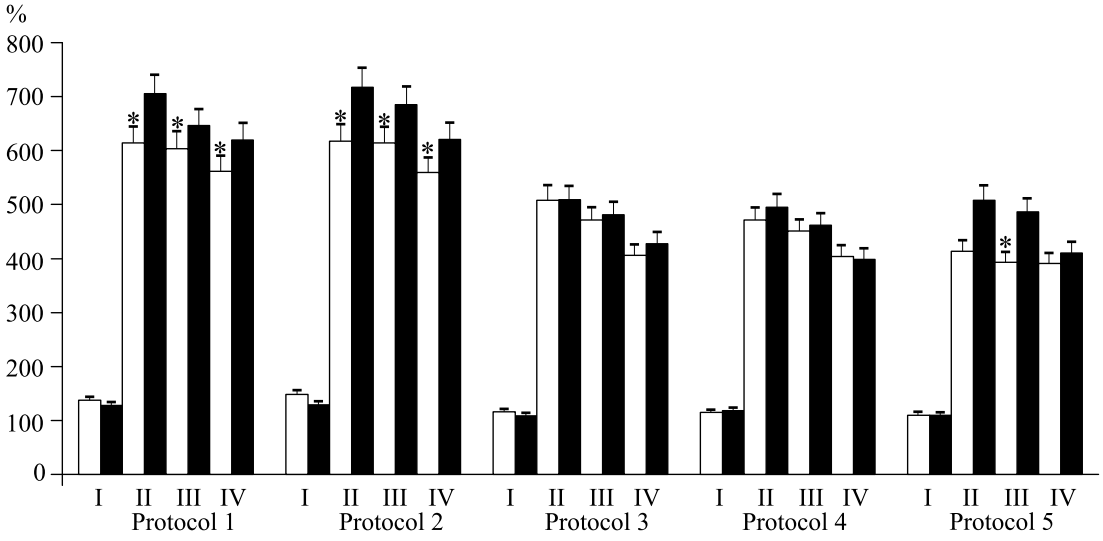

Fig. 3. EDP after IP.

on the 5th min of reperfusion was similar in the control and postconditioning group, but on the 15th and 30th min this parameter increased in the control group and remained practically unchanged in the postconditioning group. Hence, IP consisting of three 60-sec cycles produced a transient positive inotropic effect, but decelerated recovery of normal heart rate.

Thus, the effect of postconditioning on the pumping function and heart rhythm depends on the chosen postconditioning protocol. The use of several 20-sec cycles of reperfusion and ischemia had no effect on LVDP, but reduced reperfusion contracture throughout the reperfusion period. Postconditioning protocol consisting of three 40-sec cycles promoted LVDP recovery during the reperfusion period, had no effect on EDP, and decelerated recovery of normal heart rhythm. Six 40-sec cycles had no effect on LVDP and EDP, but impeded HR recovery during the reperfusion period. Postconditioning protocol consisting of three 60-sec cycles increased the force of heart contractions and reduced contracture, but these transient effects were accompanied by decelerated HP normalization.

Thus, the obtained principally new data on the effect of IP on the functional state of the myocardium are of theoretical and practical importance. These results can be useful for cardiosurgical hospitals, where artificial circulation is used during surgeries, departments and institutions studying the problems of ischemic and reperfusion damages to the heart, and pharmaceutical companies developing drugs for the treatment of cardiovascular diseases.

The study was supported by Ministry of Education and Science (grants (Nos. 2.1.1/530 and 2.1.1/211), Russian Foundation for Basic Research (grant No. 10-04-00288-a) and Federal Agency for Science and Innovations (contract 02.740.11.0714).

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