Ischemic Postconditioning and Size of Myocardial Infarction during Inhibition of Norepinephrine Reuptake

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We studied the effect of inhibition of norepinephrine reuptake during the reperfusion period on the size of infarction zone after focal myocardial ischemia and under conditions of ischemic postconditioning. In groups 1 and 2, 30-min occlusion of the left coronary artery followed by 120-min reperfusion was performed. In groups 3 and 4, ischemia was followed by ischemic postconditioning (six 10-sec occlusions alternating with 10-sec reperfusions). Ringer solution (1 ml, groups 1 and 3) and desipramine (0.8 mg/kg, groups 2 and 4) were injected intravenously at the beginning of reperfusion. The area of myocardial infarction in group 1 was 32.0±3.1% of the area of the risk zone; in groups 2, 3, and 4 the corresponding value was 46.1±3.4% (p=0.006), 22.2±2.6% (p=0.028), and 50.3±3.1% (p=0.018), respectively. It was shown that inhibition of norepinephrine reuptake in the early reperfusion period after ischemia increased myocardial injury and abolished the protective effect of ischemic postconditioning.

Key Words: *myocardial ischemia; ischemic postconditioning; norepinephrine; desipramine*

Administration of norepinephrine (NE) reuptake inhibitor desipramine before ischemia attenuates myocardial injury [10]. However, desipramine administered before reperfusion does not reduce, but even enlarges the infarction zone. This effect is apparently associated with elevated NE concentrations in myocardial interstitium [3]. Since protective mechanisms mediating ischemic postconditioning are not directly related to NE metabolism in sympathetic nerve endings in the myocardium, it was interesting to study the effect of NE reuptake inhibition during reperfusion on the size of infarction zone after local ischemia and under conditions of ischemic postconditioning.

MATERIALS AND METHODS

Experiments were performed on male Wistar rats (n=33, mean body weight 334.9±4.4 g) maintained in a vivarium under standard conditions. Experiments were conducted in accordance with the "Rules of Studies on Experimental Animals" (Supplement to the Order of the Ministry of Health of USSR, No. 755 of 12.08.1977).

The animals were subjected to tracheotomy under urethane anesthesia (2.10±0.04 mg/kg intraperitoneally). Artificial lung ventilation was conducted with a mixture of air and 20% O_2 using a Model 683 device (Harvard Apparatus). ECG was monitored during the experiment. Core body temperature was monitored with a rectal probe and maintained by heating of the operation table. Left-side thoracotomy was performed in the fifth intercostal space. After dissection of the pericardium, a nylon loop was applied around the left coronary artery at a distance of 1-2 mm below the left auricle. Both ends of the loop were introduced into the holes of a plastic occluder.

The animals were assigned into 4 groups. Occlusion (30 min) and reperfusion (120-min) were performed in animals of groups 1 and 2. Ringer solution (1 ml) and desipramine (0.8 mg/kg) were injected intravenously to group 1 rats (control; n=7) and 2 (n=7), respectively, at the beginning of reperfusion. In ani-

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mals of groups 3 (n=9) and 4 (n=10) 30-min occlusion was followed (within 10 sec) by postconditioning (six 10-sec occlusions alternating with 10-sec reperfusions) and then 120-min reperfusion was performed. Ringer solution (1 ml) and desipramine (0.8 mg/kg) were injected intravenously to rats of groups 3 and 4, respectively, at the beginning of reperfusion.

After the end of the experiment, the perfusion zone (area at risk) was determined. To this end, the coronary artery was repeatedly occluded and 2% Evans blue solution (4 ml) was injected intravenously. The animals were euthanized by overdose of the anesthetic drug. The heart was removed and unstained area of the left ventricle (area at risk) was excised and cut into 1-mm slices. The slices were incubated in 1% triphenyltetrazolium at 37°C for 20 min. Intact myocardium stained red and infarction area remained unstained. After incubation, myocardial slices were scanned, and the ratio of infarction area to area at risk was calculated.

During the analysis of experimental data presented as $M\pm m$, nonparametric statistics (Mann–Whitney test with 0.05 significance level) was used.

RESULTS

In group 1, the infarction area after 30-min ischemia followed by 120-min reperfusion constituted 32.0±3.1% of area at risk (Fig. 1). In group 2, desipramine increased infarction area to 46.1±3.0% of area at risk (p=0.006). Ischemic postconditioning (group 3) reduced infarction area to 22.2±2.6% (p=0.028 in comparison with the control). Desipramine combined with ischemic postconditioning in reperfusion period increased the infarction area to 50.3±3.1% (p=0.018 in comparison with group 3), which did not significantly differ from that in group 2 (p>0.05).

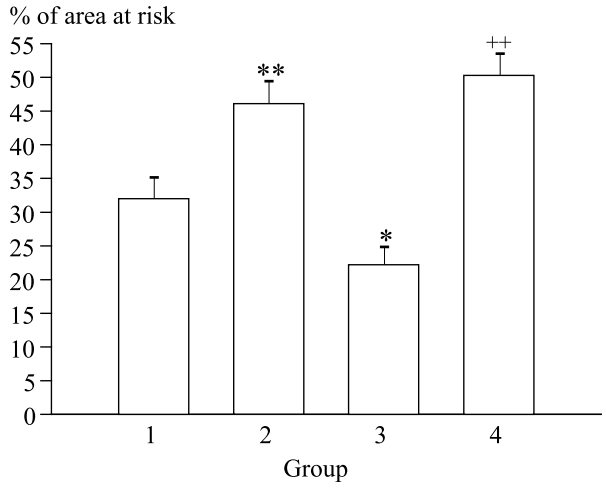

Fig. 1. Infarction area in the blockade of NE reuptake under conditions of myocardial ischemia and ischemic postconditioning. *p<0.05, **p<0.01 versus group 1, ++p<0.02 versus group 3.

It is known that during ischemia, massive NE release occurs from sympathetic nerve endings in myocardial interstitial space [1,8]. NE accumulation in the interstitium is characterized by phasic pattern. At the beginning of ischemia, NE accumulation is determined by exocytosis and is limited by reuptake. After 20-min ischemia, reversal of NE reuptake mechanism leads to further rapid and substantial NE accumulation in the myocardial interstitium [12].

Substantial accumulation of NE in myocardial interstitium is a factor contributing to its dose-dependent damage [11]. Thus, prevention of NE accumulation in the interstitial space during ischemia could reduce myocardial damage. Indeed, administration of NE reuptake blocker desipramine before ischemia reduced myocardial infarction area. The authors attributed this beneficial effect to the decreased NE accumulation because of suppression of ischemia-induced reversed NE reuptake [10].

It is also known that normal reuptake recovers quickly during reperfusion and NE concentration in the interstitium effectively decreases [1]. It can be assumed that NE reuptake blockade during this period will contribute to ischemic damage. We have previously shown that NE reuptake blockade in the reperfusion period increases infarction area. This is probably due to the excessive NE accumulation in the interstitium after rapid recovery of normal NE reuptake mechanism [3]. Ischemic preconditioning does not abolish the aggravating effect of NE reuptake blockade during the reperfusion period [2].

Despite similar result (reduction of myocardial damage), the mechanisms of natural cardioprotection (ischemic pre- and postconditioning) have some differences [14]. Since cardioprotective mechanisms in ischemic postconditioning are not directly related to NE exchange in sympathetic nerve endings of the myocardium, we can assume that ischemic postconditioning should provide necessary protection for myocardium even against the background of NE reuptake blockade in the reperfusion period.

In our experiments ischemic postconditioning in group 3 animals reduced the infarction area, which agreed with published reports [7,14]. However, this protective effect was completely abolished by NE reuptake inhibitor injected at the reperfusion onset.

Activation of free-radical oxidation is thought to be the leading mechanism of myocardial damage during ischemia-reperfusion [4]. Limitation of this process largely determines the cardioprotective mechanism of ischemic postconditioning [7]. At the same time, a positive correlation between NE levels and the formation of free hydroxyl radicals in the

myocardial interstitium was presented as an explanation for mediator cytotoxicity [9]. It is believed that generation of reactive free radicals is determined by 3,4-dihydroxyphenylglycolaldehyde, an NE metabolite produced in the reaction catalyzed by monoamine oxidase A, rather than NE. Under conditions of oxidative stress, free radicals are generated in reactions with this substrate [5].

NE accumulation in the interstitium can be apparently regarded as a universal damaging factor for myocardium, and its effects cannot be prevented by activation of natural protective mechanisms (ischemic pre- and postconditioning) under conditions of NE reuptake blockade.

The results confirm potential risk of NE reuptake inhibition in the early reperfusion period after myocardial ischemia. It is no coincidence, that administration of tricyclic antidepressants blocking NE reuptake is associated with increased risk of acute myocardial infarction [6].

Thus, inhibition of NE reuptake in the early reperfusion period after ischemia leads to increased myocardial injury and abolishes the protective effect of ischemic postconditioning.

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