Ischemic heart conditioning has been shown to protect the organ against ischemia/reperfusion injury. Animal studies have revealed that the heart can also be conditioned by non-ischemic procedures, namely physical exercise and tachycardia. Long and short term endurance training, sprint training, resistance or interval training and even one bout of exercise induce cardiac preconditioning, which is manifested by a reduction in post ischemia/reperfusion infarct size, ventricular arrhythmia and improved heart function. Several factors contribute to the exercise-induced heart preconditioning, among which the most important can be: increased activity of the anti-radical defense system, opioids, interleukin-6, nitric oxide, ATP dependent potassium channels, heat shock protein 72 and sphingosine-1-phosphate. A few studies have also shown that one bout of exercise in patients with stable angina increases tolerated workload. According to some data obtained in swine and dogs, stimulated tachycardia before ischemia/reperfusion reduces the infarct size. Future studies are needed to fully clarify the mechanisms responsible for exercise- or tachycardia-induced heart preconditioning against ischemia/reperfusion. It may lead to the development of new treatment modes of the disease.

**Key words:** heart preconditioning, heart infarction, heart reperfusion, cardioprotection, exercise, tachycardia, ischemia/reperfusion, nitric oxide

**INTRODUCTION**

Collateral circulation in the coronary arterial bed is very poor. In most cases, it is not able to provide blood to the area of the myocardium supplied by an occluded artery. As a result, blockade of blood flow in the coronary artery results in necrosis of the myocardium down below the occlusion. It is, therefore, obvious that early reconstitution of the blood flow is necessary to maintain the ischemic portion of the myocardium vivid. However, restoration of the blood flow in the ischemic area produces a complex of dangerous consequences, including exacerbation of cardiomyocyte injury and worsening of the heart condition manifested by an increase in the infarct size, impaired contractility, ventricular arrhythmias, microvascular dysfunction, myocyte necrosis, impaired healing of the infarct area, developing heart failure and other symptoms (1, 2). The complex of symptoms is called ‘ischemia/reperfusion (I/R) injury’ (1). Therefore, it is understandable that much effort has been put to developing means to prevent/reduce these changes. The pharmacological heart conditioning was used with no fully satisfactory results (3-5). A new opening was brought out by Murry et al. (6) in experiments performed on dogs. In one group of the animals, 4 cycles of 5 min occlusion of the left circumflex coronary artery separated by 5 min reperfusion were applied. Next, the vessel was occluded either for 40 min or for 3 hours. In the control group, 40 min and 3 h occlusions were also conducted but without the proceeding procedure of 5 min occlusions/reperusions. Four days later, the infarct size was measured in each group. The pre-infarction procedure reduced the infarct size after 40 min ischemia to only 25% of that seen in the respective control group. It did not affect the infarct size after 3 h occlusion of the artery, as compared to the group not receiving the procedure. The researchers called the phenomenon seen in the first group ‘preconditioning with ischemia’. Now, the procedure is usually referred to as ‘ischemic preconditioning’. In the next years much work has been done to elucidate the phenomenon and its clinical application. It can be induced before I/R (ischemic preconditioning), during evolving infarction (ischemic postconditioning) or after infarction at the onset of reperfusion (ischemic postconditioning) (7, 8). It was further established that the same procedure applied not only to another coronary artery of the same heart (9, 10) but also to distant arteries in other organs induces heart preconditioning (11, 12). The latter procedure was called remote heart preconditioning (12, 13). It can be easily applied in a non-invasive way, e.g. using a blood pressure cuff on an arm or a leg (14). Acute ischemic heart conditioning is a promising, adjunctive clinical procedure in the protection against ischemia/reperfusion injury (15). Recently, a few contributions have been published indicating some beneficial effects of daily, prolonged, repeated remote ischemic conditioning. The procedure has been shown to improve the endothelial function and microcirculation both in healthy subjects (16) and in patients with chronic heart failure (17). It also attenuates the left ventricular remodeling after myocardial infarction (18), reduces the blood pressure, the plasma level of NT-proBNP and tissue plasminogen activator (t-PA) in patients with chronic ischemic heart failure (19, 20). Another study on patients with mild ischemic heart failure
demonstrated an elevation in the left ventricular ejection fraction, heart rate variability, 6-minute walk distance and a reduction in the plasma level of BNP after the procedure (21).

Another mode of heart conditioning is non-ischemic conditioning provided by physical exercise or by tachycardia. Exercise induces cardioprotection both indirectly and directly. The indirect action requires a long-term endurance type of training that normalizes plasma lipid level and composition, improves insulin sensitivity, reduces blood pressure and body weight (22-24). Direct action means that exercise preconditioning the heart either by the blood-borne/neural factors or through increasing heart workload. Numerous reports have shown that direct cardioprotection may be exerted by different types of exercise: prolonged endurance training, a few day endurance exercise or even one bout of exercise. Some reports have also revealed that resistance or interval training induces heart preconditioning. It has been calculated, basing on the available data, that exercise reduces the infarct size by 34% on average (range 19 – 78%) as compared to non-exercising controls (25).

The current study is devoted to direct heart preconditioning by exercise or by tachycardia. Firstly, we present data on the cardioprotective effect of different types of muscular exercise. Secondly, factors known to mediate the phenomenon are discussed. Next, sections on cardioprotective effect of tachycardia and on studies in humans are included. It is not a systematic review but rather a descriptive overview of the problem. The literature was searched via PubMed in the electronic MEDLINE databases.

EFFECT OF DIFFERENT TYPE OF EXERCISE ON HEART PRECONDITIONING

The methods used to investigate this problem could be applied exclusively to animal experiments. The results available point at two factors of the preconditioning action of exercise: intensity and duration. The intensity above 55% VO$_{2}$max was usually employed (26), whereas time of exercise varied a lot. In the literature concerning the problem of exercise-induced preconditioning (or cardioprotection) the word ‘training’ is somewhat misleading since even a three-day exercise is often called ‘training’. Exercise physiologists would oppose using the word ‘training’ in such a case. Training is usually regarded as a procedure which leads to a measurable increase in function as well biochemical and/or structural changes in the body (27, 28). Exercise repeated for a few days does not produce any noticeable effects. It should rather be considered as pre-training adaptation (or habituation) to training conditions. However, since the authors used the word ‘training’ we will follow it as below. Generally, the experimental protocols used in the studies on the cardioprotective action of exercise were similar. The animals were exercised and then after a selected time of post-exercise recovery the hearts were incised, perfused $ex$ $vivo$, I/R procedure was performed and desired parameters were studied. If the procedure was different it will be indicated. The data about type and duration of training are collected in Table 1.

Table 1. Type and duration of training employed in studies on exercise - induced cardioprotection.

<table>
<thead>
<tr>
<th>Type of training</th>
<th>Animal</th>
<th>Duration of training</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Endurance training</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>running</td>
<td>rat</td>
<td>20 weeks</td>
<td>32</td>
</tr>
<tr>
<td>running</td>
<td>rat</td>
<td>12 weeks</td>
<td>33</td>
</tr>
<tr>
<td>running</td>
<td>rat</td>
<td>12 weeks</td>
<td>37</td>
</tr>
<tr>
<td>running</td>
<td>rat</td>
<td>10 weeks</td>
<td>40</td>
</tr>
<tr>
<td>running</td>
<td>rat</td>
<td>8 weeks</td>
<td>58</td>
</tr>
<tr>
<td>swimming</td>
<td>rat</td>
<td>8 weeks</td>
<td>34</td>
</tr>
<tr>
<td>swimming</td>
<td>rat</td>
<td>7 weeks</td>
<td>35</td>
</tr>
<tr>
<td>voluntary wheel running</td>
<td>mouse</td>
<td>4 weeks</td>
<td>37</td>
</tr>
<tr>
<td>running</td>
<td>rat</td>
<td>10 days</td>
<td>39</td>
</tr>
<tr>
<td>running</td>
<td>mouse</td>
<td>7 days</td>
<td>36</td>
</tr>
<tr>
<td>running</td>
<td>rat</td>
<td>5 days</td>
<td>62</td>
</tr>
<tr>
<td>running</td>
<td>rat</td>
<td>1 or 3 days</td>
<td>41, 42, 78</td>
</tr>
<tr>
<td>running</td>
<td>rat</td>
<td>3 days</td>
<td>43, 44, 78</td>
</tr>
<tr>
<td><strong>Resistance training</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12 repetitions/set, 4 sets/day</td>
<td>rat</td>
<td>4 weeks</td>
<td>47</td>
</tr>
<tr>
<td>as above</td>
<td>rat</td>
<td>12 weeks</td>
<td>48</td>
</tr>
<tr>
<td><strong>Interval training</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 min run, 16 and 60 m/min</td>
<td>rat</td>
<td>11 – 16 weeks</td>
<td>54</td>
</tr>
<tr>
<td>1 min run/1 min walk</td>
<td>rat</td>
<td>6 weeks</td>
<td>52</td>
</tr>
<tr>
<td>2 min run, 95 – 100% VO$_{2}$max/</td>
<td>rat</td>
<td>5 days</td>
<td>53</td>
</tr>
<tr>
<td>2 min run, 65 – 75% VO$_{2}$max/</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>3 min run, 50% VO$_{2}$max</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 min run, 85 – 90% VO$_{2}$max/2 min walk</td>
<td>mouse</td>
<td>5 weeks</td>
<td>55</td>
</tr>
</tbody>
</table>

LONG-TERM ENDURANCE TRAINING

According to the above, only results obtained after training lasting more than one week will be discussed in this section. Since many data unanimously indicate that prolonged endurance training exerts a strong cardioprotective effect against I/R injury (26, 29-31), only a few examples are presented here. More information is also included in the section ‘Mechanism of exercise-induced preconditioning’.

The longest training protocol lasted for > 20 weeks in rats. The exercise intensity on a treadmill was increasing gradually.
up to 35 m/min and up to 20 min daily. The hearts were removed and perfused. The left anterior descending coronary artery (LADA) was occluded for 60 min and then reperfused for 2 hours. The training reduced the infarct size by 25% (32). The same group of authors trained rats for 12 weeks and found the infarct size to be reduced by 34% (33). Swimming training for 8 weeks reduced the infarct size by 24% (34). Interesting data were obtained by (35), who trained rats for 7 weeks (swimming) and thereafter induced myocardial infarction in vivo. Four weeks later, in the trained group the scar area was 1.6-fold smaller, arteriolar density was 1.7-fold higher, and left ventricular shortening fraction was 1.9-fold higher in the trained compared to sedentary animals. In mice, a 7-day treadmill training reduced the infarct size by 34% (36), whereas 4-week voluntary wheel running by 22% (37). Long term endurance exercise was shown to improve cardiac function after I/R in rats, which was manifested by better recovery of contractile function, lower diastolic stiffness, greater efficiency of work, and greater extracellular calcium responsiveness than hearts from sedentary post-I/R rats (38). In another study, rats were trained for 10 days, 60 min daily with increasing speed. Twenty four hours after the last exercise bout, hearts were subjected to I/R (25 min/120 min). Exercise protected against infarction and arrhythmia, and preserved coronary flow (39). Different approach was employed in rats by (40). After a 10-week endurance training program, the animals were anesthetized, mechanically ventilated, and the chest was opened by thoracotomy. Coronary occlusion was achieved by a ligature around the left coronary artery and I/R was evoked (20/10min). The exercise-trained animals maintained higher peak systolic blood pressure throughout I/R compared with the untrained ones.

SHORT-TERM ENDURANCE TRAINING

All studies using short-term endurance training showed reduction in the post I/R infarct size (26, 29-31). Here are some examples of the experiments reporting functional advantages of this type of training. Taylor et al. (41) run rats for 100 min at 20 m/min, 6° upgrade, on 1 or 3 consecutive days. Twenty four hours later they studied cardiac function using isolated perfused heart. Applied global ischemia lasted for 17 min and subsequent reperfusion for 30 min. Both 1- and 3-day exercise accelerated post I/R recovery of cardiac output. Locke et al. (42) exercised rats for 60 min at 30 m/min also on 1 or 3 consecutive days. Twenty four hours after exercise, hearts were subjected to I/R (30/30 min). Animals exercising for 3 days showed recovery of the left ventricular developed pressure after I/R faster than the sedentary ones. Lennon et al. (43) exercised rats for 3 consecutive days, 60 min daily, at workload of 55 or 75% VO2max. Three 2–3 min rest periods were introduced during each bout of exercise. Twenty four hours after the third exercise bout, I/R (20/30min) was applied. I/R markedly decreased cardiac output and cardiac work in both groups but the reductions were less pronounced in the trained one. No difference was recorded in the level of cardioprotection between the two exercised groups. However, it should be mentioned that the rats were habituated to running for 5 days at 55% VO2max, which might have interfered with the final effect of exercise. French et al. (44) exercised rats for 3 days at ~70% VO2max, 60 min/daily, 30 m/min. The exercise reduced the infarct size, apoptosis and necrosis of cardiomyocytes and prevented calpain activation as compared to the sedentary group. Importantly, the cardioprotective effect of short-term endurance training was comparable to that achieved by the long-term one (26, 31).

RESISTANCE EXERCISE

Resistance exercise training is highly recommended in the prevention of metabolic and circulatory disorders (45, 46). Unfortunately, only two studies on the cardioprotective effect of this type of training have been found. In one study (47), rats were adapted to resistance exercise for 4 weeks and in the other study (48) for 12 weeks, according to the same protocol. After the last exercise bout in the training program the hearts were excised and perfused. The LADA was occluded for 40 min and then reperfused for 80 min. The 4-week training had no effect on the infarct size, left ventricular developed pressure and coronary flow compared to the sedentary I/R group (47). The 12-week training reduced the infarct size and allowed maintenance of higher coronary flow and left ventricular developed pressure over the period of ischemia and reperfusion compared to the untrained controls (48). It suggests that only long-term resistance training exerts cardioprotection against I/R.

INTERVAL TRAINING

Generally speaking, in an anaerobic type of interval training, short-term efforts of high intensity are interspersed with short-term efforts of low intensity. This training is mainly used to improve anaerobic capacity. In an aerobic type of interval training, repeated short-term high intensity aerobic exercise bouts are interspersed with very brief rest periods. It shortens the time of training necessary to produce results obtained after long, continuous training (49-51). A few reports are available on the cardioprotective action of the aerobic type of interval training in different species. Rats were trained for 6 weeks. Five 1 min runs at 75 m/min, 15° grade, were interspersed with 1 min walks at 20 m/min. Another group of rats was trained at the same time at 20 m/min. Forty eight hours after the last exercise, I/R (20/30min) was performed. The training improved the mechanical function of the heart, which was manifested by higher left ventricular developed pressure and lower left ventricular end diastolic pressure during reperfusion than in the sedentary rats (52). Rahimi et al. (53) exercised rats for 5 days, 6 × 2 min at 95 – 100% VO2max. The exercise was interspersed with 5 × 2 min run at 65 – 5% VO2 max and 3 min run at 50% VO2max. The I/R procedure (30/90min) was applied on day 1, 7 and 14 after the last exercise bout. The training reduced the infarct size: by 50% after one day, by 35% after 7 days and by 19% after 14 days (the latter was insignificant). Training did not affect the occurrence of arrhythmia. Bowles et al. (54) compared cardioprotective effects of 11 – 16 week endurance training of low intensity (20 m/min, 0% grade, 60 min/day), moderate intensity (30 m/min, 5° grade, 60 min/day) and interval training (10 bouts of alternating 2 min runs at 16 and 60 m/min, 5° grade). The percent recovery of cardiac output after ex vivo total 25 min ischemia was: 36 in the control group, 61.2 in low intensity trained, 68.1 in moderate intensity trained and 73.2 in interval trained rats. The differences between the trained groups were not significant, indicating that the decisive factor was the training itself and not the type of training. Interval training proved also to be substantially effective in I/R reduction in mice maintained on high-fat diet for 8 weeks. Then, one group underwent 5 week high-intensity interval training (daily, 10 × 4 min at 85 – 90% VO2max, 25° inclination, interspersed by 2 min active rest), whereas the other group was kept sedentary. The training reduced I/R infarct size by 47% as compared to high fat diet sedentary rats (55). These data clearly indicate that the interval training is a potent stimulus in the development of the cardioprotective phenotype.
A SINGLE BOUT OF EXERCISE

Domenech et al. (56) instrumented dogs with a snare on the anterior descending coronary artery. After healing, the animals were exercised acutely, 5 times × 5 min. In one group of the animals the aorta was occluded 10 min after exercise, whereas in the other one a day after exercise. The occlusion lasted for 1 hour and subsequent reperfusion for 4 hours. The exercise reduced the infarct size by 78% in the first group and by 46% in the other group, indicating that only one bout of exercise is able to induce preconditioning and that it decreases rather fast. These effects could not be explained by changes in collateral flow to the ischemic region. It should be stressed, however, that it was the interval exercise. In another report by the same group (57), the exercise reduced the infarct size by 56% after 1 hour coronary artery occlusion. Very recently, a study was published in which the effect of a single maximal exercise was compared to an 8-week endurance training in a group of rats. Three days after the last exercise in the training program, I/R procedure (30/60 min) was performed. The left ventricle developed pressure was similar in both groups at the basal conditions. However, after I/R the pressure in the trained group was over 70% higher than after a single bout of exercise (58).

ISOLATED CARDIOMYOCYTES

Kang et al. (59) subjected rats to a 6-week endurance training. Thereafter, the cardiomyocytes were isolated and subjected to hypoxia/reoxygenation. The training protected the cells against apoptosis produced by the procedure. This is an important information indicating that the cells themselves acquire preconditioning phenotype during training.

PERSISTENCE OF EXERCISE PRECONDITIONING

Yamashita et al. (60) showed that the exercise-produced cardioprotection is biphasic. They exercised rats acutely for 25–30 min on a treadmill moving with the speed of 27–30 m/min. Thereafter, in situ LADA was ligated for 20 min and then reperfused. After 0.5, 48 and 60 hours of reperfusion the infarct size was smaller than in sedentary animals, whereas the reduction was not observed after 3, 24, 36 and 72 hours of reperfusion. It was paralleled by changes in the myocardial activity of manganese superoxide dismutase, the reactive oxygen species scavenger. Interestingly, a biphasic cardioprotection was also reported after exercise in dogs (56). In humans, remote ischemic preconditioning protects against ischemia-reperfusion injury of the endothelium also in a biphasic manner (61). In a study by Lennon et al. (62) rats were subjected to a 5-day endurance training and allowed to rest for 1, 3, 9 or 18 days. At those periods of time the hearts were subjected to I/R (20.5/30 min). The exercise-trained animals showed higher relative cardiac output and cardiac work at 30 min of reperfusion and on day 1, 3, and 9 after training. It remains an open question how the mechanism inducing cardioprotection could be maintained active for such a long time after the exercise bout.

MECHANISMS OF EXERCISE-INDUCED HEART PRECONDITIONING

Prolonged cardiac ischemia results in several biochemical changes in the myocardium, including a reduction in ATP level, accumulation of hydrogen ions, increased level of free calcium in the cytosol, increased formation of reactive oxygen species (ROS) and activation of calpain (calcium dependent protease). Subsequent reperfusion further increases the production of ROS, overloading with free calcium, activation of caspase-3 and calpain, and opening of the mitochondrial permeability transition pores (mPTP). These complex factors induce mitochondrial injury and in consequence lead to cardiomyocyte death (63, 64). Several factors have been shown to participate in the exercise-induced cardioprotection (Table 2). However, it seems to be a multifactorial phenomenon rather than a consequence of an action of a single factor. Factors contributing to exercise-induced preconditioning could be divided into two groups, namely the extracardiac (neural and blood-borne) and the intracardiac ones.

**EXTRACARDIAC FACTORS**

**Neural factors**

Some data indicate that in case of remote ischemic preconditioning, stimulation of the sensory nerve endings in the ischemic area participates in the heart conditioning. However, it is not certain which level of the spinal cord is mandatory for the reflex. We are not aware of any studies on the skeletal muscle originated neural pathways mediating the exercise-induced cardioprotection. However, the contractile activity changes the metabolic environment in the muscles. It could activate sensory receptors within the muscle and the signal would be transferred to the heart as it happens during remote ischemic preconditioning. The fact that higher workloads are needed to produce cardioprotection would support such an assumption.

**Blood-borne factors**

The skeletal muscles are very active secretory tissue. The bioactive compounds secreted by the muscles are called myokines. In humans, over 300 compounds of likely bioactive

<table>
<thead>
<tr>
<th>Extracardiac factors</th>
<th>Intracardiac factors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myokines</td>
<td>Mitochondria</td>
</tr>
<tr>
<td>Opioids</td>
<td>Radical oxygen species (ROS)</td>
</tr>
<tr>
<td>Autonomic nervous system</td>
<td>ATP-sensitive potassium channels (KATP)</td>
</tr>
<tr>
<td></td>
<td>Heat shock protein 72 (HSP72)</td>
</tr>
<tr>
<td></td>
<td>Nitric oxide (NO)</td>
</tr>
<tr>
<td></td>
<td>Sphingosine-1-phosphate (likely)</td>
</tr>
<tr>
<td></td>
<td>Tachycardia</td>
</tr>
</tbody>
</table>

Table 2. Factors mediating the exercise-induced cardioprotection.
properties have been found to be secreted by the muscles but only a few have been examined closely. Myokines exert broad effects both on the muscles themselves and on other organs of the body. According to the available data, acute exercise increases secretion and blood concentration of the following myokines: interleukin-6 (IL-6), interleukin-7 (IL-7), interleukin-15 (IL-15), decorin, follistatin-like 1 (FSTL1), brain-derived neurotrophic factor (BDNF), angiotensin-like protein 4 (ANGPTL4), and MCP-1 (66, 67). So far, only a role of IL-6 in the acute exercise-induced heart preconditioning has been studied. In wild mice, exercise (running for 3 days, 18 m/min, 60 min daily) protected against I/R induced arrhythmia and necrosis and reduced the infarct size. In IL-6 knockout mice the exercise did not reduce the post I/R infarct size but augmented arrhythmia (68). Serum concentration of IL-6 increased several times after exercise only in wild mice. Serum IL-6 receptor level increased in both groups after exercise but it was much higher in the wild than in the IL-6 knockout animals (68). That strongly points to IL-6 as the principal mediator in exercise-induced cardioprotection. These data are in line with the results showing involvement of IL-6 in ischemic preconditioning (69, 70). FSTL1, another myokine, was shown in mice and swine to accelerate heart infarct healing (71). No data are available on its acute cardioprotective effect. We are certainly only at the beginning of investigating the role of myokines in exercise-induced heart preconditioning. However, it seems to be a very promising area of research.

Opioids

Opioids (opiates) were shown to be involved in ischemic heart preconditioning (72, 73). Some data indicate that opioids are also involved in the exercise-induced cardioprotection, acting both as blood-borne and myocardial factors. In a study by Michelsen et al. (74) healthy young men underwent four cycles of 2 min high-intensity exercise with 3 min intervals of low intensity exercise. Five minutes after the last exercise bout, the blood was collected and plasma dialysate was prepared. Next, rabbit hearts were isolated and perfused with the dialysate for 25 min and washed out for 5 min. The left marginal coronary artery (in rabbit, it is equivalent to LADA in rats and dogs) was occluded for 40 min and it was followed by reperfusion for 120 min. The dialysate reduced infarct size from 60 to 35% of the area at risk and improved the left ventricular developed pressure. In the next experiment, naloxone (a nonspecific opioid receptor antagonist) was added to the dialysate. Naloxone abolished the cardioprotective effects of exercise. This would indicate that opioids released during exercise are present in the dialysate and they could be responsible for the cardioprotective effect of exercise. The source of opioids remains unclear. Unfortunately, the level of plasma opioids was not measured in the study. Dickson et al. (75) investigated this problem in rats. Naltrexone (a nonspecific antagonist of opioid receptors) was administered twice: on day 4 and 5 before the test exercise. Rats exercised for 20 min at 25 m/min. I/R was generated in isolated perfused heart (20 min/3 h). The exercise reduced the infarct size by 50% as compared to nonexercised rats. This effect was absent 5 days after exercise. Blockade of the opioid receptors thoroughly removed exercise-induced cardioprotection. Immediately after exercise, the mRNA expression of all three opioid receptors (δ, κ and µ) and mRNA expressions of their precursors increased several-fold, almost disappearing 8 hours later. The authors concluded that opioids mediate, at least in part, the cardioprotective effect of exercise. In another study (76) rats were exercised for 4 days, 60 min daily at ~70% of VO2max. Twenty four hours after the last exercise bout, I/R was performed in vivo by LADA closing for 30 min and subsequent reperfusion for 60 min. 10 min prior to I/R, selective or nonselective opioid receptor antagonists were administered intravenously. The exercise reduced the infarct size by about 30% and this effect was thoroughly abolished by the blockade of both non-selective or selective δ-opioid receptor antagonist. Neither the blockade of µ- nor κ-opioid receptors affected the infarct size after exercise. These data clearly indicate that the exercise-induced preconditioning was mediated by δ-opioid receptors. A role of opioids in cardioprotection produced by long-term training was also studied (77). Rats were exercised for 12 weeks at 60% VO2 max. Naloxone was administered before each bout of exercise. Fourty eight hours after the last exercise bout the LADA was ligated and rats were recovering for 7 days. The training reduced the infarct size over 3 times, the effect being prevented by naloxone. Treatment with morphine right before I/R also had a cardioprotective effect, but only a mild additive effect of training and morphine was observed. The role of δ-opioid receptors in exercise-induced cardioprotection was at the same time described by Miller et al. (78), who used a 3-day training, 30 min at 30 m/min. Naltrindole, a selective δ-opioid receptor blocker was administered on each day before exercise. Twenty four hours after the last exercise bout the rats were anesthetized and in vivo I/R procedure was performed. The LADA was ligated for 50 min and then reperfused for 120 min. The exercise reduced the infarct area by over 50% as compared to the sedentary group. This effect of exercise was partially prevented by the blockade of δ-opioid receptors. The left ventricle mRNA of proenkephalin was elevated immediately after exercise and 120 min later. The exercise did not affect the ventricular mRNA expression of δ-opioid receptors. Neither the protein expression of Leu-enkephalin nor δ-opioid receptors were affected by the exercise. Also the serum concentration of enkephalin remained stable. The blockade of δ-opioid receptors prevented necrosis but not apoptosis induced by I/R. They hypothesized that both the blood borne and the intracardiac opioids were likely to participate in the exercise-induced preconditioning.

Autonomic nervous system

Muscular exercise increases the activity of the adrenergic nervous system. It induces tachycardia and increases inotropy during exercise (27, 28). It has been shown that the adrenergic system is involved in cardioprotection (25, 37). This is discussed in the ‘Nitric oxide’ section.

Mitochondria

As already indicated, mitochondria play a key role in I/R heart injury (63, 64). The available data clearly indicate that endurance training also induces the development of a mitochondrial phenotype that is resistant to I/R injury. Other major features of the phenotype include decreased reactive oxygen species (ROS) production, increased tolerance to high calcium level, reduced rate of opening of the mitochondrial permeability transition pores (mPTP) and elevation in the content of antiapoptotic proteins (64, 79-82). Endurance training induces changes in the expression of a number of mitochondrial proteins. Many of them participate in ATP production. However, no direct relationship was reported between the latter group of proteins and cardioprotection (64, 83). The mPTP play an important role in cardioprotection afforded by ischemic preconditioning. The pores are present in the inner mitochondrial membrane and are closed. Their opening at the onset of reperfusion results in the appearance of a cascade of events leading finally to cell death. Ischemic
conditioning prevents opening of the pores (84, 85). Long-term endurance training was shown to increase resistance of the mitochondrial permeability transition pore opening during I/R in rats (86, 87) and mice (88). Also short-term (5 days) training reduced mPTP opening in I/R in rats. Interestingly, the training did not affect the heart contractile function and coronary flow and LDH release (89), suggesting a specific response of mPTP to the training. Unfortunately, we are not aware of any data regarding a response of mPTP to acute exercise. The data on mitochondrial ROS and ATP-sensitive potassium channels are included in the sections below.

**Reactive oxygen species**

ROS are claimed to play the major role in I/R injury (90, 91). Therefore, studies were undertaken to elucidate a role of the antioxidative defense system in the exercise-induced cardioprotective phenotype. ROS are produced both in mitochondria and in the cytosol (80, 82, 91). The major components of the antioxidative defense system include such enzymes as superoxide dismutase (SOD), catalase, and glutathione peroxidase (82, 91). The data collected by (30) and (91) indicate that both short-term and long-term exercise training increases the activity of the heart MnSOD. Low-intensity exercise does not seem to affect the cardiac activity of MnSOD (82). Most studies failed to show an elevation in the activity of catalase and glutathione peroxidase in the myocardium after exercise (26, 82, 91). The data regarding the effect of training on the activity of myocardial glutathione reductase are not uniform (26, 82). Yamashita et al. (60) used antisense oligodeoxyribonucleotide against MnSOD. The treatment prevented exercise training-induced reduction in the infarct size, which was confirmed by French et al. (44). Hamilton et al. (92) showed that antisense oligodeoxyribonucleotide prevents arrhythmia during I/R. On the other hand, Lennon et al. (93) observed no influence of antisense oligonucleotides on the development of myocardial stunning after I/R. They concluded that different mediators may protect against stunning and infarction. Taylor and Starnes (94) administered MPG, a free radical scavenger, before exercise and preformed I/R procedure 24 hours after exercise. The compound did not affect the heart external work output. It remains to be established whether the cytosolic isoform of SOD participates in the exercise-induced cardioprotection (30).

**ATP-sensitive potassium channels**

There are two groups of the ATP-sensitive potassium channels: sarcolemmal (sKATP) and inner mitochondrial membrane (mKATP) (95, 96). The channels are closed when the level of intracellular ATP is adequate. The reduction in the level of intracellular ATP is adequate. The reduction in the level of intracellular ATP takes place during ischemia, which results in the opening of sKATP channels and subsequent leakage of potassium ions from cardiomyocytes. This, in turn, prevents entry of calcium ions into the cells, thus preventing calcium overload. Decreased availability of ATP also leads to opening of mKATP channels, which may protect mitochondria against calcium overload and subsequent damage during I/R (97, 98). Brown et al. (33) blocked sKATP channels with a compound termed HMR 1098 and mKATP channels with 5-hydroxydecanoic acid in endurance trained rats. Blockade of sKATP channels completely abolished the training-acquired cardioprotection, whereas blockade of mKATP channels did not provide any effect. The results would strongly indicate that sKATP channels are responsible for cardioprotection, which, however, has not been confirmed yet. As already mentioned, in dogs, acute high intensity exercise both prior to and 24 h before coronary occlusion induced early and late cardiac preconditioning. The early effect was removed by the blockade of mKATP channels (56). In a subsequent study, according to the same protocol, late cardiac preconditioning by acute exercise was also mediated through mKATP channels (99). Very recent data revealed that remote ischemic preconditioning also reduced infarct size and arrhythmia, the effect being abolished by the blockade of mKATP channels (100). Certainly, by far more studies are necessary to elucidate the role of KATP channels in exercise-induced cardioprotection.
iNOS. Thirty min later, the LADA was ligated for 25 min. In the control group, exercise reduced the severity of arrhythmia, increased survival rate and increased the activity of the enzyme in the left ventricle after 24 h but not after 48 h of recovery. The administration of ammoguanidine prevented the cardioprotective effects of exercise (107). In another study on dogs, a selective iNOS inhibitor (AEST) and nonselective NOS inhibitor (L-NNAME) were used. Exercise markedly diminished ventricular arrhythmia 24 h after I/R. This effect was substantially reduced by L-NNAME administered before exercise as well as by AEST given prior to coronary artery ligation. This would indicate that NO is cardioprotective both early and late after exercise (108). However the results obtained in rats are in odds with the results obtained in dogs (109). It should be added that in trained rats, coronary endothelial cells but not cardiomyocytes were shown to be responsible for the eNOS-dependent cardioprotection (110).

Ceramide and sphingosine-1-phosphate

Ceramide and sphingosine-1-phosphate (S1P) are key bioactive sphingolipids (111, 112). I/R increases the content of ceramide in the perfused rat heart, the effect being partially prevented by ischemic preconditioning (113). In the rat, acute exercise was shown to reduce the heart content of ceramide (114). Ceramide activates apoptosis of cardiomyocytes after I/R (115-117). However, no data regarding the effect of exercise on post-I/R content of ceramide in the myocardium are available. S1P was repeatedly shown to exert a very powerful cardioprotective action against I/R injury. Exogenous S1P increases viability of cardiomyocytes incubated under hypoxic conditions and reduces the infarct size in the isolated, perfused rat heart after I/R. It also mediates the beneficial effect of ischemic pre- and postconditioning in the heart subjected to I/R. Formation of S1P in the heart is catalyzed by the enzyme sphingosine kinase 1 and its catalabolism is catalyzed by the enzyme sphingosine-1-phosphate lyase. The reduction in the activity of sphingosine kinase 1 or knocking out its gene eliminates a cardioprotective effect of ischemic preconditioning in mice. Also knocking out the sphingosine-1-phosphate lyase gene has a very potent cardioprotective effect against I/R injury in the mouse heart (116-118). It is important to note that the extracellular S1P binds to S1P receptors present on the plasma membrane both of the mother cells (autocrine action), neighboring cells (paracrine action) and on remote cells, reaching them by the blood plasma (119, 120). Acute exercise was shown to increase plasma S1P in untrained healthy humans (121) and it would increase the exercise-induced cardioprotective potential of the compound. However, the late post-infarction plasma S1P concentration was found to be reduced in humans (122, 123) and rats (124). The plasma concentration of S1P remained unchanged during percutaneous coronary artery intervention (125), but it was elevated during reperfusion after percutaneous artery occlusion (126). However, as in case of ceramide, no data are available regarding the influence of exercise on post-I/R content of S1P in the heart.

HUMAN STUDIES

For obvious reasons, no direct data are available on the cardioprotective effect of acute exercise in humans. It should be mentioned, however, that Michelsen et al. (74) showed in healthy humans that a transferable, cardioprotective factor is released to the plasma during exercise. A few reports are also available indicating the cardioprotective effect of acute muscular activity against cardiac ischemia in patients with coronary heart disease. Bilinska et al. (127) studied patients with stable angina. The patients performed a symptom-limited exercise test, followed by the second exercise test (as above) either on day 1, 2, 3 or 4 of recovery. The patients showed improved exercise time and other heart variables on day 1 and 2 after exercise. The improvements decreased on day 3 and disappeared on day 4 of recovery. This observation clearly showed that the first exercise preconditioned the patients’ hearts to the second exercise bout. These data were confirmed by Lambiase et al. (128) and Crisafulli et al. (129). Lambiase et al. (128) examined two groups of patients with stable angina due to isolated and discrete stenosis within LADA. One group of patients performed 3 maximal exercise tests (basal, 15 and 90 min later) and recovered for 14 days. Then, each patient had elective percutaneous coronary intervention (PCI) comprising two balloon inflations lasting for 180 s. The second group of patients also performed 3 exercise bouts as above but 24 h later had two additional tests separated by 15 min rest, and then 4 h later they underwent PCI. The tests performed 24 h after the first 3 tests showed improved performance and reduction in ventricular ectopic frequency. During the first and the second balloon inflation the peak ST elevation was markedly reduced as compared to the first group. This indicates that in the second group, the first sets of exercise produced late preconditioning and this effect was manifested both during exercise and PCI. Crisafulli et al. (129) examined a group of patients with stable angina. First, they performed a maximal exercise-test and then, the subsequent maximal tests 30 min and 2 days later. In these two subsequent tests (30 min and 2 days later), the onset of ischemia was delayed, and the allowed workload, heart rate and cardiac output were higher as compared to the respective data in the first exercise-test. Only exercise after the 2-day recovery elevated myocardial contractility and stroke volume. However, it should be kept in mind that the exercise test in humans with stable angina lasted until symptoms of ischemia appeared. Therefore, it is difficult to evaluate whether silent ischemia contributed to the preconditioning. Certainly, many more studies are needed to fully investigate the exercise-induced preconditioning in humans and its possible clinical usefulness.

TACHYCARDIA-INDUCED PRECONDITIONING

Some data clearly indicate that tachycardia per se induces heart preconditioning. Koning et al. (130) subjected open-chest pigs to 30 min ventricular pacing at 200 beats/min followed by 15 min normal sinus rhythm and then produced myocardial infarction by a 60-min coronary artery occlusion. The pacing resulted in a reduction in the infarct size by ~30%. 30 min pacing immediately preceding the occlusion without intervening sinus rhythm reduced the infarct size by ~40%. The reductions in the infarct size were abolished by pretreatment with the KATP channel blocker glibenclamide. The results would suggest that tachycardia itself, without ischemia, provides considerable acute preconditioning. That cardioprotective effect of tachycardia was also confirmed in dogs (131). Anesthetized dogs were induced with 5 periods of tachycardia (213 ± 12 cycles/min), each of 5 min duration, and 5 min of intervening periods at control heart rate. Next, the LADA was occluded for 60 min and reperfused for 4.5 hours. The tachycardia reduced the infarct size by 46% with respect to the group without preconditioning. This effect was not due to changes in the collateral flow. During tachycardia, myocardial interstitial adenosine showed an approximately twofold increase. Blockade of adenosine receptors with 8 phenyltheophylline, before or after preconditioning tachycardia, reverted its protecting effect but it did not modify infarct size in non-preconditioned dogs. No differences in cytosolic or particulate protein kinase C activity or translocation of α-, β-, γ-, and ζ- protein kinase C isozyme were
observed between preconditioned and non-preconditioned dogs. It was concluded that tachycardia, in the absence of ischemia, mimics the preconditioning effect of ischemia in the dog, the effect being mediated by adenosine. It should be added that several other factors might contribute to tachycardia-induced preconditioning. Tachycardia increases the usage of glucose (132, 133) and free fatty acids by the heart (134). It induces numerous changes in the metabolism of sphingolipids (135), glycerolipids (136) and major components of the lipolytic complex (137) in the ventricles. Endurance training in rats reduces glycolysis and elevates glucose and palmitate oxidation both before and after an insult of ischemia. The latter changes have been collectively called a ‘protective metabolic phenotype’ (138). All these changes in the metabolic milieu could affect heart susceptibility to I/R. Moreover, cardiomyocytes belong to a group of cells empowered with the mechanism of mechanotransduction. The elevation in mechanical tension induces the release of bioactive compounds by the cells. They act in either autocrine or paracrine way (139, 140). It is likely that increased mechanical tension during tachycardia also releases the local factor(s) contributing to preconditioning. It is a tenet that the increasing exercise intensity is paralleled by heart rate elevation. As already mentioned, higher exercise intensity is needed to produce cardioprotection. It may be hypothesized that exercise-induced tachycardia may participate in the generation of preconditioning by exercise.

CONCLUSIONS AND PERSPECTIVES

Ischemic heart conditioning is a well-recognized, clinically applicable phenomenon. In its shadow, numerous data have been published indicating that heart preconditioning may be induced in a nonischemic way, namely by muscular exercise or tachycardia. Exercise-induced heart preconditioning is a very interesting phenomenon. It can be produced by different types of exercise, such as long term or short term endurance training, acute endurance exercise, resistance training, interval training and even by one bout of exercise. Cardiac preconditioning reduces the infarct size, arrhythmia and stunning after ischemia/reperfusion. Surprisingly, even cardiomyocytes isolated from the hearts of trained rats show a higher level of resistance against hypoxia/reoxygenation. Exercise-induced preconditioning is a multifactorial process involving both extracardiac and intracardiac factors. The extracardiac factors include interleukin 6, opiates and the adrenergic system. The major intracardiac factors include the antioxidant defense system, heart opiates, nitric oxide, ATP-dependent potassium channels, heat shock protein 72 and sphingosine-1-phosphate. Several reports indicate that removal of only one factor prevents exercise-induced cardioprotection. Therefore, it remains unclear how all the factors cooperate at the same time. Experimental tachycardia has also been found to reduce the infarct size. Tachycardia-induced cardioprotection is probably mediated by adenosine. All information concerning exercise and tachycardia-induced cardioprotection has been obtained in experiments performed on animal models. In healthy humans, the presence of a transferable, cardioprotective factor in the plasma has been observed after exercise. Some data obtained in humans with stable angina indicate that one exercise bout increases the time of allowable exercise workload of next bouts. Ischemic heart conditioning is produced by a non-physiological procedure, whereas exercise and tachycardia-induced preconditioning is generated by physiological stimuli. They usually act along since exercise is accompanied by heart rate acceleration. No acute clinical application of the two stimuli has been reported so far. However, they may represent a considerable potential. It is tempting to claim that in case of exercise-inducing preconditioning, the principal, but yet not recognized stimulus, originates from the contracting skeletal muscles thus ‘taking care’ of the heart. The stimulus would activate all other mediators of cardioprotection. IL-6 would be a candidate to play such a role though it exerts many extra-heart effects. However, the existence of another, yet unidentified, myokine acting specifically on the heart cannot be excluded. Its identification and synthesis would probably enable cardiac preconditioning without exercise before procedures on patients’ coronary arteries.

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