Blocked Production of Thermal Shock Proteins Prevents the Cardioprotective Effect of Adaptation to Exercise

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The resistance of isolated rat heart to ischemia and reperfusion increases after adaptation to exercise (swimming, 30 sessions 1 h/day), which correlates with accumulation of HSP70 cytoprotector proteins in the myocardium. Quercetin blockage of HSP70 production during adaptation prevents the development of adaptation defense of the heart. It was hypothesized that the accumulation of HSP70 in the myocardium is an important mechanism of local adaptation defense of the heart.

Key Words: *adaptation to exercise; heart; ischemia and reperfusion; HSP70; quercetin*

Cardioprotective effects of the adaptation to exercise are largely determined by the formation of defense mechanisms of the heart [8,9]. This is manifested in increased resistance of isolated heart of a trained animal to ischemia and reperfusion [1,10], toxic concentrations of calcium and catecholamines [4], and thermal shock [2]. The nature of these local defense mechanisms is unclear. The resistance of cell structures to injury may be increased due to accumulation of thermal shock cytoprotective HSP70 proteins [11]. Warming of animals, which induces the production and accumulation of HSP70 in the myocardium, increases heart resistance to ischemia and reperfusion [6,12], to postischemic increase in Ca^{2+} content in cardiocytes [5], and to Ca^{2+}-paradox [8,11].

We analyzed the time course of the resistance of an isolated rat heart to ischemia and reperfusion and the time course of HSP70 accumulation in the myocardium during adaptation to exercise (swimming) and evaluated the effect of blockade of HSP70 production on cardiac resistance induced by adaptation.

MATERIALS AND METHODS

Experiments were carried out on 76 male Wistar rats weighing 250-350 g. Adaptation was attained by forced swimming in water (32°C) for 45 days 5 times a week (30 sessions). During the first two weeks, the duration of swimming was increased from 15 to 60 min a day, after which the animals swam for 60 min every day. The animals were taken into experiment after 5, 10, 15, and 30 days of training, i.e., during "immediate adaptation", at the transitional stage, and after formation of stable adaptation to exercise [3,9]. The resistance of the myocardium to ischemia and reperfusion was evaluated in isolated heart perfused by Langendorff. Changes in cardiac contractility were assessed from isotonic contractions measured with a TD-112S pick-up and recorded with an RM-6000 polygraph (Nihon Kohden). Ischemic and reperfusion injuries were induced by complete blocking of coronary blood flow for 15 min, after which perfusion was resumed and cardiac contractions were recorded for 15 min of reper-

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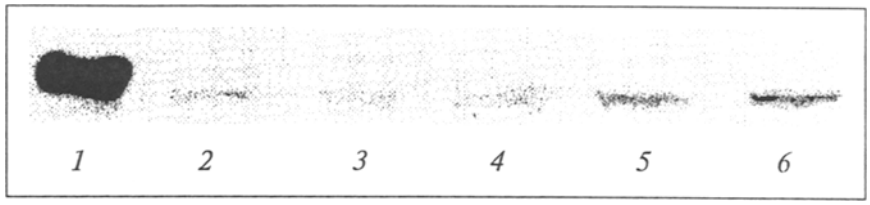

Fig. 1. Time course of HSP70 accumulation in the cytoplasmatic fraction of the myocardium during adaptation to exercise. Western blots: *1*) HSP70 standard; *2*) control; *3-6*) 5, 10, 15, and 30 days of adaptation, respectively.

fusion. The extent of dysfunction was assessed from the amplitude of contractions and heart contracture. The content of HSP70 in the myocardium was measured by Western-blot analysis. Activation of HSP70 production was prevented by quercetin, a transcription blocker of the hsp70 gene [7], which was injected intraperitoneally in a concentration of 5 mg/kg during the entire adaptation period starting from the first day twice a week 30 min before training. Control rats were injected with the drug according to the same regimen.

RESULTS

The time course of contractile function of an isolated heart during adaptation indicates that stability to ischemia and reperfusion develops only by the end of training (after 30 sessions of adaptation).

Table 1 shows the effect of a complete course of adaptation to exercise and quercetin on the resistance of the contractile function of an isolated heart to ischemia and reperfusion. Ischemia decreased the amplitude of contractions of isolated hearts to the zero in all groups of animals. Recovery of the amplitude of contractions after cessation of ischemia (during reperfusion) was more rapid in "adapted" hearts than in the control. After a 5-min reperfusion, this value in adapted hearts was 2.5 times higher than in the control. Ischemic contractures in adapted hearts developed after 15 min of ischemia, which is later than in the control, where contractures of 0.25 mm were observed after 10 min of ischemia. During reperfusion the contractures in adapted hearts were 1.7-fold smaller than in the control (Table 1).

The development of heart resistance to ischemia and reperfusion during adaptation correlated with the time course of HSP70 content in the myocardium. HSP70 accumulated after 15 sessions of adaptation, reaching the maximum after 30 sessions, i.e., after formation of stable resistance of the myocardium to ischemia and reperfusion (Fig. 1). In adapted rats with HSP70 production blocked by quercetin (adaptation+quercetin) the resistance of the myocardium to ischemia and reperfusion did not increase (Table 1). Reperfusion contracture of the myocardium in these animals was virtually the same as in the control. After 10 min of reperfusion it was 2.1 times greater than in adapted animals given no quercetin injections. Injection of quercetin to control rats according to the same protocol did not affect the ischemic and reperfusion disorders of the contractile function of isolated heart (quercetin).

TABLE 1. Amplitude of Contractions and Contracture of an Isolated Rat Heart in Ischemia Followed by Reperfusion ($M \pm m$, n=8)

Variant	Initial level	Ischemia, min			Reperfusion, min		
		5	10	15	5	10	15
Amplitude of heart contractions, mm							
Control	2.04±0.09	0	0	0	0.44±0.18	0.38±0.14	0.36±0.13
Adaptation	2.25±0.10	0	0	0	1.12±0.20*	0.97±0.16*	0.96±0.16*
Quercetin	2.27±0.14	0	0	0	0.72±0.14	0.51±0.15	0.51±0.15
Adaptation+quercetin	2.34±0.10	0	0	0	0.43±0.21**	0.38±0.21**	0.35±0.20**
Heart contracture, mm							
Control	0	0	0.25±0.23	0.69±0.34	1.30±0.13	1.18±0.12	1.19±0.12
Adaptation	0	0	0	0.23±0.19	0.81±0.18*	0.69±0.16*	0.69±0.15*
Quercetin	0	0	0	0.44±0.38	1.10±0.22	1.10±0.22	1.03±0.24
Adaptation+quercetin	0	0	0	0.42±0.30	1.51±0.22**	1.45±0.23**	1.38±0.23**

Note. $p<0.02$: *vs. the control, **vs. adaptation.

These results indicate that heart resistance to ischemia and reperfusion develops with accumulation of cytoprotective HSP70 proteins in the myocardium. The HSP70 production blocker quercetin prevents this effect of adaptation. Therefore, activation of production and accumulation of HSP70 in the myocardium is an important factor in the formation of local mechanism of adaptation at the heart level.

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