Effect of *Rhodiola rosea* on the Resistance of Isolated Heart from Stressed Rats to Ischemic and Reperfusion Damage

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 123, No. 5, pp. 514-517, May, 1997
Original article submitted December 25, 1995

Experiments on isolated hearts from rats pretreated with *Rhodiola rosea* and subjected to combined cooling and immobilization show that this adaptogen improves the resistance of the myocardium to ischemic and reperfusion damage and exerts a membrane-stabilizing effect.

Key Words: *cooling; creatine phosphokinase; contractility; isolated heart; Rhodiola rosea*

In 1957, H. Selye pointed out the possibility of damage to the heart induced by cold stress [7]. Moreover, it is well known that fall of outdoor temperature considerably increases the incidence of myocardial infarction [6,8]. Under real conditions cooling is usually combined with the influence of other stress factors which can potentiate its adverse effects. However, the effect of cooling in combination with other stress factors has not yet been studied.

Our previous experiments showed that acute cooling of albino rats decreases myocardial contractility *in vitro*, while pretreatment with *Rhodiola rosea* extract (RRE) prevents these disturbances [1]. However, possible mechanisms of the cardioprotective effects of *Rhodiola rosea* detected by us under conditions of cold and psychoemotional stress and in acute myocardial ischemia [3] remain unclear.

The aim of the present study was to evaluate the effect of the adaptogen RRE on the resistance of isolated heart from rats exposed to a combination of low temperature and immobilization to the damaging effect of total ischemia and reperfusion.

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MATERIALS AND METHODS

Experiments were carried out on 36 Wistar male rats weighing 200-250 g. The rats were divided into 3 groups, 10-12 animals in each. Group 1 rats were stress-control. Group 2 rats were daily treated with adaptogen preparation RRE for 8 days in a dose of 1 ml/kg *per os*. Control group 3 comprised intact rats. A combination of acute cooling and immobilization was used as a model of stress: the rats were immobilized in supine position for 4 h at 5°C.

Further experiments were carried out on rat hearts isolated by the Langendorff method [5]. The hearts were perfused with Krebs—Henseleit solution saturated with carbogen at 37°C. After a 15-min adaptation to perfusion [5], the hearts were subjected to a 45-min total normothermal ischemia followed by a 60-min reperfusion. In all series, the contractile function of perfused heart was assessed in isotonic regime under a constant load of 5 g, and coronary flow was measured. The amplitude of contractions and changes in the heart diastolic length (contracture) were calculated. Diastolic length was measured from the zero line 0.5 mm below diastolic fragment of the contraction—relaxation curve. The damage to cardiomyocyte sarcolemma was assessed from creatine phosphokinase (CPK) activity in perfusate using com-

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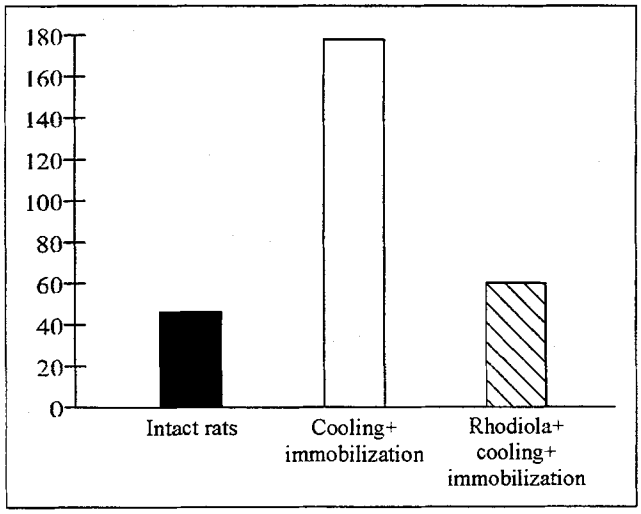

Fig. 1. Increase in diastolic length of isolated rat heart by the 45th min of total ischemia (% of preischemic value). All differences from the preischemic value are significant at $p<0.05$.

mercial kits (Sigma). CPK activity was expressed as percentage of its initial value (100%). The data were processed statistically using the Student's t test.

RESULTS

Figure 1 shows that after a 45-min total ischemia of hearts isolated from intact animals their diastolic length decreased by 52% of the initial value, which is indicative of ischemic contracture of the myocardium. In stressed animals, the increase in diastolic length was much more pronounced and constituted 178% on average. These findings suggest that hearts from stressed rats are more susceptible to ischemia. This also follows from the fact that in 70% of cases reperfusion of hearts from intact animals led to restoration of rhythmic contraction during the first 5 min, while in hearts from stressed rats rhythmic activity was recovered only in 50% of experiments.

A stable decrease in the amplitude of heart contraction during reperfusion was noted in both intact rats and stressed controls; however, it was more pronounced in the myocardium of stressed animals (Fig. 2, *a*). This was attended by an increase in myocardial contracture, which was also more pronounced in stressed rats (Fig. 2, *b*). Impaired contractility and defective diastole during reperfusion resulted in a considerable reduction of coronary flow in these two experimental series (Fig. 2, *c*).

TABLE 1. Perfusate CPK Activity During Reperfusion After Total Ischemia of Isolated Heart (in % to Preischemia Level)

Group	Reperfusion	
	5 min	60 min
Control	281±113	437±108
Cooling+immobilization	256±51	858.8±200
RRE+cooling+immobilization	227±64	78±13

During the first minutes of reoxygenation, there were no considerable differences in CPK activity of perfusate between the control and stressed rats (Table 1). After a 60-min reperfusion, CPK activity in intact rats rose only by 1.5 times, while in animals subjected to cooling and immobilization this parameter increased by 3.5 times ($p<0.05$). These data suggest that combined action of acute cooling and immobilization sensitizes rat myocardium to reperfusion damage.

The course of RRE-preconditioning prevented stress-induced potentiation of ischemic contracture (Fig. 1) and protected the heart from reperfusion damage. For instance, the diastolic length of the heart from preconditioned rats subjected to stress was 90% shorter than in stressed controls and practically did not differ from that in intact animals. Moreover, by the 5th min of reperfusion, the amplitude of heart contraction in these animals was considerably higher than in both control groups and remained at this level throughout the reoxygenation period (Fig. 2, *a*).

In adaptogen-pretreated animals, reperfusion caused no defective diastole and did not potentiate contracture in comparison with the period of total ischemia. Moreover, we observed a decrease in diastolic tension during reperfusion compared with the control group (Fig. 2, *b*).

Coronary flow in hearts from adaptogen-pretreated animals subjected to stress did not differ considerably from that in intact animals and after a 60-min reperfusion surpassed that in hearts from stressed controls (Fig. 2, *c*). The maintenance of sufficient coronary flow is apparently a factor weakening reperfusion damage to cardiomyocytes. This is also confirmed by lower CPK activity in the corresponding perfusate samples (Table 1).

Thus, our findings show that cold stress in combination with immobilization promote the development of stable ischemic and reperfusion contracture of isolated heart and damage to the cardiomyocyte plasma membrane. Preconditioning with RRE prevents contractile disturbances induced by total ischemia and reperfusion and reduced the CPK release in hearts isolated from stressed rats.

The positive effect of RRE on myocardial contraction and relaxation is most likely determined by modulation of energy metabolism [2,4]. This assumption is confirmed by our previous investigations which demonstrated stimulation of ATP resynthesis in the myocardium of rats treated with RRE before exposure to cold.

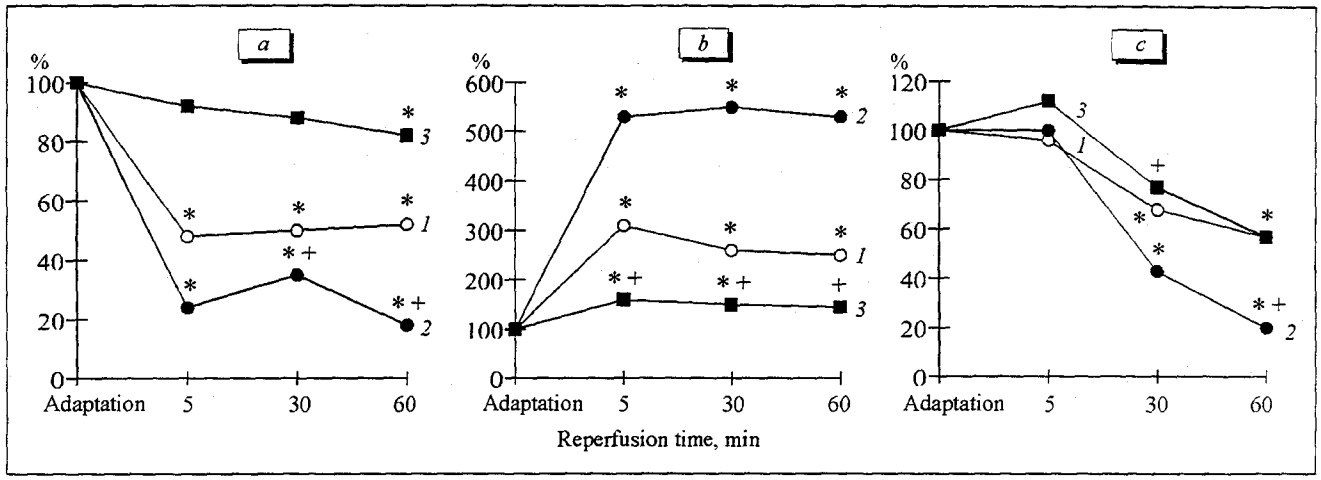

Fig. 2. Contractile activity of isolated rat heart during reperfusion after total ischemia (preischemic values are taken as 100%). *a*) amplitude of contractions; *b*) diastolic tension; *c*) coronary flow; *1*) intact rats; *2*) cooling+immobilization; *3*) *Rhodiola*+cooling+immobilization. $p<0.05$: *compared with preischemic values, +between curves *2* and *3*.

The cardioprotective effect of RRE verified by reduced release of CPK from cardiomyocytes can be attributed to a direct membrane-stabilizing effect of the adaptogen.

The study was partially supported by the Russian Foundation for Basic Researches.

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