Do Endogenous Ligands of Peripheral μ- and δ-Opiate Receptors Mediate Antiarrhythmic and Cardioprotective Effects of *Rhodiola rosea*?

L. N. Maslov, Yu. B. Lishmanov, A. V. Naumova, and T. V. Lasukova

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Oral administration of *Rhodiola rosea* extract to rats (3.5 ml/kg, 8 days) prevents reperfusion arrhythmias and elicits a protective effect in experiments on isolated heart. Experiments with naloxone and ICI 174,864 suggest that the antiarrhythmic effect of *R. rosea* extract is mediated through activation of μ-opiate receptors in the myocardium. The cardioprotective effect of this extract is not associated with opiate receptors.

Key Words: *μ- and δ-opiate receptors; arrhythmias; isolated heart; adaptogens*

It has been previously shown that extract of *Rhodiola rosea*, a natural adaptogen, exhibits cardioprotective and antiarrhythmic activity *in vivo* [2,4]. Moreover, we showed that repeated administration of this preparation induces accumulation of opioid peptides in tissues [2-4], while preliminary injection of the nonselective opiate receptor (OR) blocker naloxone abolishes the antiarrhythmic effects of *R. rosea* [3]. These data suggest that the above-mentioned effects of *R. rosea* are mediated through activation of cardiac OR of their endogenous ligands.

In light of the above and because the search for new means for preventing ischemic and reperfusion damage to the myocardium is an important medical problem, we studied opiatergic mechanisms of the antiarrhythmic and cardioprotective effects of *R. rosea* on isolated perfused rat heart.

MATERIALS AND METHODS

Experiments were carried out on 94 Wistar rats weighing 200-250 g and adapted by repeated oral administration (3.5 ml/kg for 8 days) of an officinal *R. rosea* extract. The dose and the scheme of administration were chosen on the basis of our previous data on antiarrhythmic effect of the preparation *in vivo* [2]. In some experimental series, 15 min prior to heart isolation the rats were intravenously injected with naloxone (Sigma) in a dose of 0.5 mg/kg (sufficient for blockade of μ- and δ-OR [5]) or ICI 174,864 (Chiron Mimotopes Peptide System), a blocker of δ-OR, in a dose of 2.5 mg/kg [5]. Intact animals served as the control.

The heart was isolated and perfused by Langendorff as described previously [1,7]. The heart was adapted to perfusion conditions for 20 min and then, after recording the initial parameters, total normothermal ischemia was modeled by interrupting the perfusion with Krebs—Henseleit solution for 45 min followed by 60-min reperfusion. Electrodes for electrocardiogram recording were positioned on the right atrium and left ventricle. Recording and computer-assisted processing of ECG were performed using an UBF 4-03 biopotential amplifier (Pushchino). The total percentage of ventricular arrhythmias and the percentage of ventricular fibrillations were estimated in each group of isolated hearts for a 60-min reperfusion period. Damage to cardiomyocytes was assessed by creatine phosphokinase (CPK) activity in the perfusate measured with NAC-activated CK 47-

Department of Experimental Cardiology, Institute of Cardiology, Siberian Division of the Russian Academy of Medical Sciences, Tomsk

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20 kits (Sigma). If baseline CPK activity by the end of 20-min perfusion under conditions of normoxia exceeded 7.0 μmol NADH/min×liter, the heart was excluded from experiments in accordance with previously published recommendation [8]. The data were processed statistically using the Student's *t* test.

RESULTS

Repeated administration of *R. rosea* extract completely prevented the development of reperfusion ventricular arrhythmias *in vitro* (Table 1). For instance, arrhythmias were noted in 62% control hearts isolated from intact rats, while no cases of arrhythmia were observed over the 60-min reoxygenation period in hearts isolated from *R. rosea*-treated rats and, more important, the most severe rhythm disturbance, ventricular fibrillation, was absent in these hearts. These findings confirm our previous data on antiarrhythmic activity of *R. rosea* extract *in vivo* [2,3].

Blockade of μ- and δ-OR in rats treated with *R. rosea* extract completely abolished the increased resistance of hearts isolated from these animals to reperfusion arrhythmias (Table 1). It should be noted, that injection of naloxone to nonadapted rats had practically no effect on the occurrence and type of reoxygenation arrhythmia. Unlike naloxone, the selective antagonist of δ-OR ICI 174,864 had no effect on the antiarrhythmic activity of *R. rosea*. This suggests that endogenous ligands of δ-OR are not involved into adaptive protection of the heart against arrhythmogenic influences.

It can be hypothesized that protective antiarrhythmic effect of *R. rosea* is mediated through specific activation of μ-OR by endogenous ligands. This hypothesis is confirmed by the fact that *R. rosea* extract increases the content of Leu-enkephalin in rat myocardium [9].

It is likely that the effect is mediated through cardiac μ-OR. If it is not the case, and extracardiac OR are also involved, we should admit a possibility of rapid (within 15 min from injection of OR agonist to isolation of the heart) modulation of the neurohumoral regulation of the heart. Moreover, these changes should be potent enough to induce considerable functional rearrangements of the myocardium persisting under *in vitro* conditions and providing improved cardiac resistance to reperfusion arrhythmias. Such an assumption is highly improbable. However, localization of μ-OR mediating the antiarrhythmic effects of *R. rosea* can be determined only in additional experiments, where agonists should be added into perfusate.

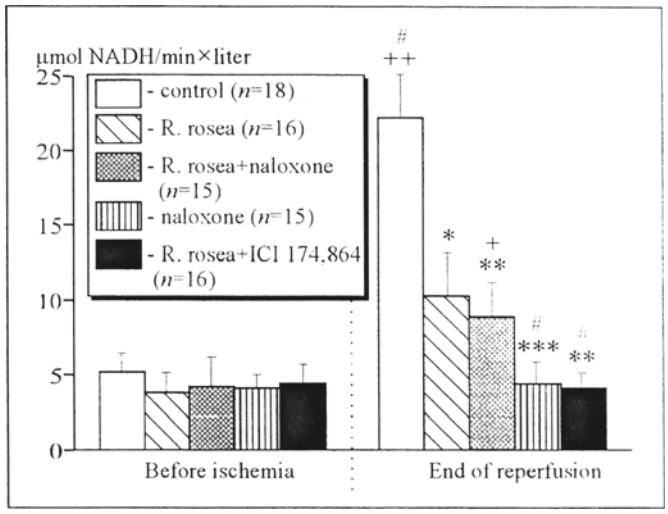

Fig. 1. Activity of creatine phosphokinase in perfusate after 45-min total ischemia of isolated rat heart. *n*: number of observations. **p*<0.05, ***p*<0.01, ****p*<0.001 compared with the control; #*p*<0.05 compared with rats treated with *R. rosea* extract; +*p*<0.05, ++*p*<0.001 compared with rats injected with naloxone.

Apart from its antiarrhythmic activity, the effect of *R. rosea* extract was also observed during the postischemic period: CPK activity in the perfusate decreased 2-fold in comparison with the control (Fig. 1). Since high level of CPK in the perfusate indicates damage to cardiomyocytes [8], decreased activity of CPK in the perfusate observed in our experiments may be indicative of cardioprotective effect of *R. rosea*. We hypothesized that the cardioprotective effect of *R. rosea*, similarly to its antiarrhythmic activity, is mediated through activation of OR. However, neither naloxone, nor ICI 174,864 suppressed this protective

TABLE 1. Reperfusion Cardiac Arrhythmias after 45-Min Total Ischemia

Series	*n*	Ventricular arrhythmias	Ventricular fibrillation
Control	21	13 (62%)++	4 (19%)+
R. rosea	19	0**	0*
R. rosea+naloxone (0.5 mg/kg)	19	12 (63%)++	5 (26%)+
Naloxone (0.5 mg/kg)	19	11 (58%)++	4 (21%)+
R. rosea+ICI 174,864 (2.5 mg/kg)	16	2 (12%)*	0*

Note. *n*: number of observations; **p*<0.05, ***p*<0.01 (χ^2 test) compared with the control; +*p*<0.05, ++*p*<0.01 (χ^2 test) compared with rats treated with *R. rosea* extract.

effect (Fig. 1). Moreover, selective blockage of δ-OR with ICI 174,864 was accompanied by a considerable potentiation of the protective effect of *R. rosea*.

This phenomenon can be attributed to intrinsic cardioprotective effects of OR antagonists. Indeed, naloxone in a dose of 0.5 mg/kg reduced CPK activity in the perfusate 5-fold in comparison with the control. Similar results were obtained in experiments where the heart was perfused under conditions of hypoxia with a naloxone-containing solution [8].

Thus, our experiments suggest that preventive antiarrhythmic effect of *R. rosea* extract against modeled reperfusion arrhythmias is associated with activation of cardiac μ-OR, while the cardioprotective properties of this preparation are not mediated through the opiatergic system.

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