PHARMACOLOGY AND TOXICOLOGY

Effect of Opiate Peptide Dalargin and Des-Tyr-Dalargin on Cardiac Pump Function during Ischemia-Reperfusion

T. V. Lasukova*,, L. N. Maslov*,**, Yu. K. Podoksenov*, A. Yu. Podoksenov*, A. A. Platonov*, M. V. Ovchinnikov***, and Zh. D. Bespalova*****

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 137, No. 1, pp. 35-38, January, 2004
Original article submitted January 14, 2003

Experiments on isolated perfused rat heart showed that nonselective μ- and δ-opiate receptor agonist dalargin decreased contractility of the intact heart, but had no effect on pump function of the ischemic myocardium. Dalargin analogue des-Tyr-dalargin not binding to opiate receptors decreased contractility of intact myocardium and isolated heart exposed to 45-min total ischemia. We hypothesize that the influence of dalargin is related to activation of cardiac δ-opiate receptors, while the inotropic effect of des-Tyr-dalargin is mediated by other receptors.

Key Words: *dalargin; des-Tyr-dalargin; opiate receptors; ischemia-reperfusion*

Agonists of opiate receptors (OR) protect the heart from adverse factors [4]. Intravenous injection of D-Ala^2,Leu^5,Arg^6-enkephalin (dalargin) decreases the incidence of ventricular arrhythmias induced by coronary occlusion in rats [5,10]. Antiarrhythmic activity of dalargin surpasses that of many antiarrhythmic drugs [5,8,10]. Moreover, dalargin is the only opiate peptide approved for the use in clinical practice. It was assumed that this preparation can be used in cardiology as an antiarrhythmic drug. However, it remained unclear whether dalargin can modulate pump function of intact heart and myocardium exposed to ischemia-reperfusion. The effect of dalargin metabolites not binding to opiate receptors (*e.g.*, des-Tyr-dalargin) on myocardial contractility was poorly studied [1,2]. Published data show that metabolites of opiate peptides (OP) produce a potent biological effect [4,12].

Here we studied the inotropic effects of dalargin and des-Tyr-dalargin on intact isolated rat heart and myocardium exposed to ischemia-reperfusion.

MATERIALS AND METHODS

Experiments were performed on male Wistar rats weighing 250-300 g. The heart was removed from the thorax immediately after thoracotomy and placed in cold Krebs—Henseleit solution (4°C). After termination of spontaneous contractions a cannula was introduced into the ascending aorta. Physiological saline was delivered through the cannula. Open-circuit retrograde perfusion of the heart with standard Krebs—Henseleit solution was performed by the method of Langendorff. Heart contractility was recorded under isovolumic conditions at a constant perfusion pressure of 60 mm Hg. Cardiac contractile activity was recorded using an electromanometer connected to a latex balloon introduced into the left ventricle.

*Laboratory of Experimental Cardiology, Institute of Cardiology, Tomsk Research Center, Siberian Division of the Russian Academy of Medical Sciences, Tomsk; **Tomsk Pedagogic University; ***Laboratory of Peptide Synthesis, Russian Research-and-Production Center for Cardiology, Russian Ministry of Health, Moscow. ***Address for correspondence:*** maslov@cardio.tsu.ru. Maslov L. N.

0007-4888/04/1371-0027

The heart was adapted to normoxic perfusion for 20 min, after that the perfusion was stopped for 45 min (total myocardial ischemia) and then resumed for 30 min (reperfusion). Experiments were performed only on hearts whose initial contractility corresponded to our laboratory standards. The hearts from intact animals exposed to total ischemia and reperfusion served as the control.

We recorded heart rate (HR, bpm), left ventricular pressure (mm Hg), end-diastolic pressure (EDP, % of the initial value), maximum rates of contraction and relaxation (mm Hg/sec). Left ventricular pressure was calculated as the difference between systolic and diastolic pressures.

We used nonselective μ- and δ-OR agonist dalargin (H-Tyr-D-Ala-Gly-Phe-Leu-Arg) and its analogue not containing tyrosine (des-Tyr-dalargin, H-D-Ala-Gly-Phe-Leu-Arg). Both ligands were synthesized at the Laboratory of Peptide Synthesis (Russian Research-and-Production Center for Cardiology). Dalargin and des-Tyr-dalargin in concentrations of 137 and 177 mM, respectively, were added to the perfusate to evaluate *in vitro* effects. After 20-min adaptation to normoxic perfusion the peptide was added to Krebs—Henseleit solution and perfusion was continued for 10 min under normoxic conditions. Total ischemia and reperfusion were modeled for 45 and 30 min, respectively. The preparations were dissolved in physiological saline immediately before the experiment and added to the perfusate. The concentrations of preparations were selected taking into account the results of *in vitro* assessment of antiarrhythmic and cardioprotective activity of opiates [3,6]. The results were analyzed by Student's *t* test.

RESULTS

Dalargin and des-Tyr-dalargin decreased the strength of cardiac contractions and HR during normoxic perfusion.

After 10-min perfusion with the solution containing dalargin the strength of contractions decreased by 30% compared to the control (Fig. 1, *a*). HR decreased from 216±10 to 179±11 bpm ($p<0.05$). The rates of contraction and relaxation decreased by 2 times (Fig. 2, *a*, *b*). These changes were accompanied by a 2-fold increase in EDP (Fig. 1, *b*). Published data show that stimulation of OR decreases the amplitude of contraction and rates of contraction and relaxation of the isolated perfused heart [7]. Our previous studies showed that activation of cardiac δ-OR decreased the strength of cardiac contractions and HR and increased EDP [3]. Dalargin acts as μ- and δ-OR agonist and does not bind to κ-OR [11]. We hypothesized that the negative inotropic effect of this OP is related to activation of δ-OR, since the myocardium contains only δ- and κ-OR [9].

After 10-min perfusion with a solution containing des-Tyr-dalargin changes in myocardial contractility were similar to those observed after treatment with dalargin. By the end of perfusion the strength of contractions decreased by 30%, while EDP increased by 2 times (Fig. 1). Des-Tyr-dalargin caused bradycardia (HR decreased from 216±10 to 181±14 bpm, $p<0.05$). Under these conditions changes in the rates of contraction and relaxation were similar to those induced by dalargin (Fig. 2, *a*, *b*).

These findings suggest that dalargin and its metabolite des-Tyr-dalargin produce a negative inotropic

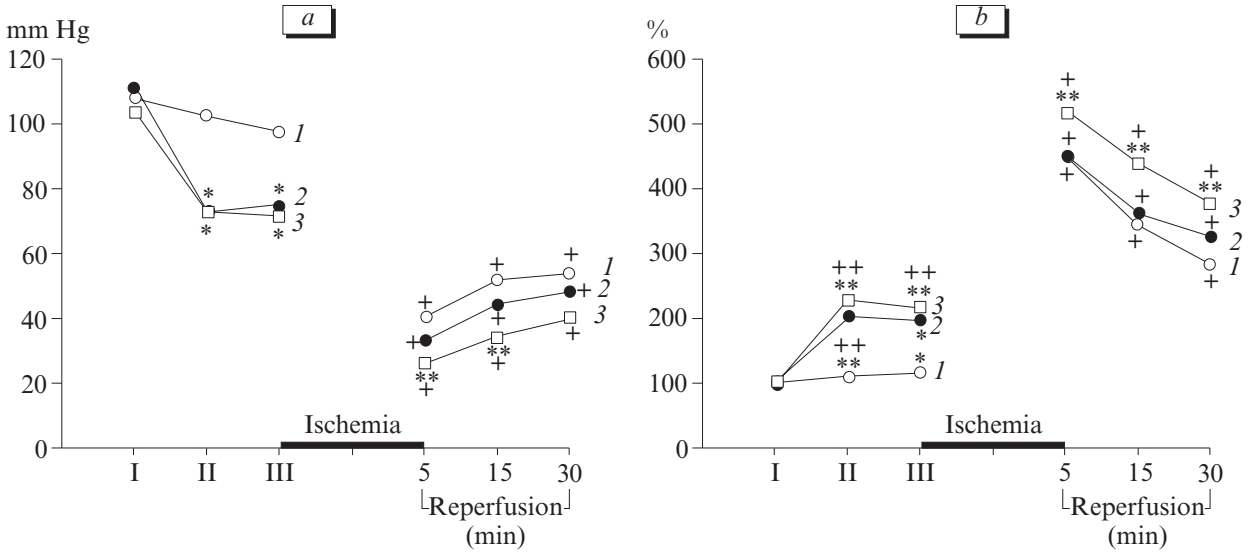

Fig. 1. Effects of dalargin and des-Tyr-dalargin on left ventricular pressure (*a*) and end-diastolic pressure (*b*). Here and in Fig. 2: adaptation, 20 min (*I*); perfusion with the preparation, 10 min (*II*); perfusion without the preparation, 10 min (*III*). Control (*1*), dalargin (*2*), and des-Tyr-dalargin (*3*). *$p<0.01$ and **$p<0.05$ compared to the control; $^{+}p<0.01$ and $^{++}p<0.05$ compared to the initial level.

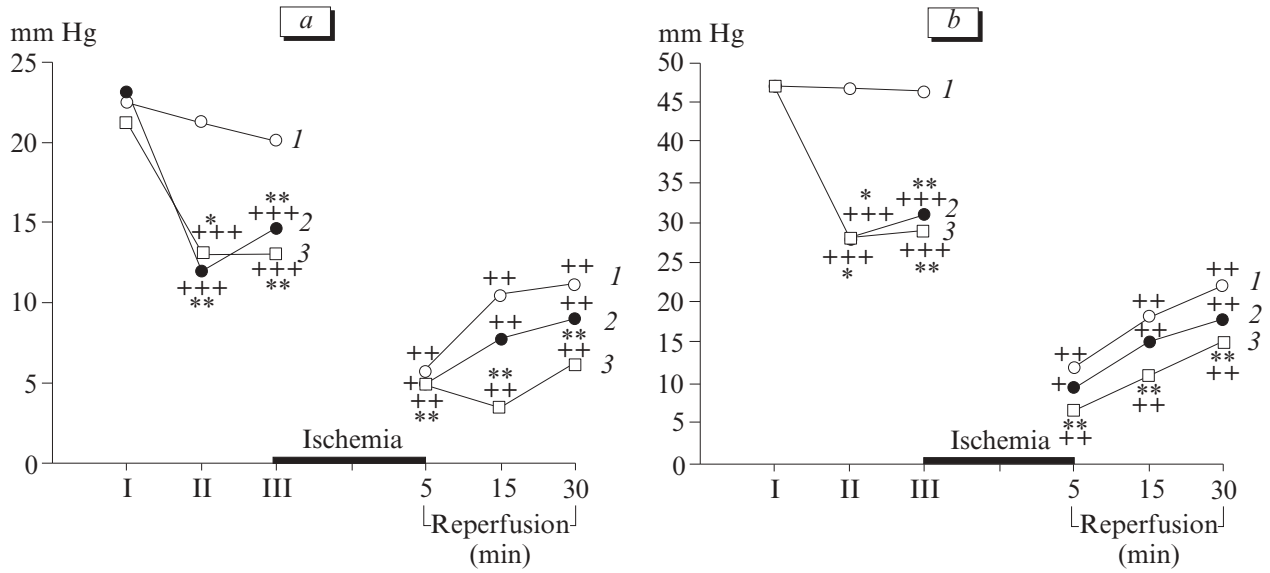

Fig. 2. Effects of dalargin and des-Tyr-dalargin (0.1 mg/liter) on the maximum rate of contraction (*a*) and relaxation (*b*) of isolated rat heart. **p*<0.01 and ***p*<0.05 compared to the control; ^{+}p<0.001, ^{++}p<0.01, and ^{+++}p<0.05 compared to the initial level.

effect on the myocardium. However, the effects of these peptides are realized via different mechanisms. The action of D-Ala^2,Leu^5,Arg^6-enkephalin is mediated by activation of OR. Similar effect of des-Tyr-dalargin is probably related to activation of nonopiate receptors.

This assumption is confirmed by the results of the present study. In control rats coronary reperfusion after 45-min ischemia was accompanied by suppression of cardiac pump function. These changes are typical of ischemia-reperfusion. Total ischemia caused cardiac arrest. However, after reperfusion the strength of cardiac contractions was brought to 50% of the initial level (Fig. 1, *a*). EDP increased by 4.5 times compared to the initial level (Fig. 1, *b*). At the beginning of reoxygenation HR was 162±15 bpm (*vs.* 225±11 bpm before ischemia, p<0.05). Bradycardia disappeared, and HR did not differ from the initial level by the 30th minute of reperfusion (200±12 bpm, p>0.05). The maximum rate of contraction decreased by 4 times at the beginning of reperfusion, but reached 50% of the initial level by the 30th minute (Fig. 2, *a*). The rate of myocardial relaxation underwent similar changes (Fig. 2, *b*).

Perfusion of the isolated heart with Krebs—Henseleit solution containing dalargin had no effect on the dynamics of contractility during reperfusion compared to the control (Fig. 1, *a*, *b*). Therefore, activation of cardiac OR with dalargin in the preischemic period did not modulate the inotropic and chronotropic function of the isolated heart during reperfusion.

In contrast to dalargin, the negative inotropic effect of des-Tyr-dalargin persisted in the postischemic period. The strength of contractions of the isolated heart during reperfusion was much lower than in control animals (Fig. 1, *a*). Postischemic EDP increased to a greater extent than in the control (by 25 %, Fig. 1, *b*). During 30-min reoxygenation HR was below the control level. By the 5th minute HR in des-Tyr-dalargin-treated rats and control animals was 124±13 and 162±15 bpm, respectively (p<0.05). The mean rates of contraction and relaxation in rats receiving des-Tyr-dalargin were 40-50% lower than in the control (Fig. 2, *a*, *b*). Therefore, des-Tyr-dalargin suppressed the pump function of the intact isolated heart and myocardium exposed to ischemia and reperfusion.

Our findings suggest that D-Ala^2,Leu^5,Arg^6-enkephalin and des-Tyr-dalargin decrease HR and produce a negative inotropic effect on the myocardium during the preischemic period. However, only des-Tyr-dalargin aggravated reperfusion bradycardia and increased the severity of contractile myocardial dysfunction. Hence, dalargin and des-Tyr-dalargin produce different effects on the myocardium. Probably, the inotropic and chronotropic effects of these peptides are realized via different mechanisms. It can be assumed that the effect of dalargin is related to activation of cardiac δ-opiate receptors, while the inotropic effect of des-Tyr-dalargin is mediated by other receptors.

This work was supported by the Russian Foundation for Basic Research (grant No. 03-0405/04).

REFERENCES

1. E. I. Kalenikova, O. F. Dmitrieva, N. V. Korobov, *et al*., *Vopr. Med. Khim.*, No. 1, 75-83 (1988).
2. N. V. Korobov, *Farmakol. Toksikol*., No. 4, 35-38 (1988).
3. T. V. Lasukova, L. N. Maslov, Yu. B. Lishmanov, and G. J. Gross, *Ros. Fiziol. Zh.*, **88**, No. 5, 568-580 (2000).

4. Yu. B. Lishmanov and L. N. Maslov, *Opiate Neuropeptides, Stress, and Adaptive Protection of the Heart* [in Russian], Tomsk (1994).
5. L. N. Maslov and Yu. B. Lishmanov, *Eksper. Klin. Farmakol*., **55**, No. 2, 25-28 (1992).
6. L. N. Maslov and Yu. B. Lishmanov, *Kardiologiya*, No. 12, 25-30 (1998).
7. C. Clo, C. Muscari, B. Tantini, *et al*., *Life Sci*., **37**, No. 14, 1327-1333 (1985).
8. M. J. Janse and A. L. Wit, *Physiol. Rev*., **69**, No. 4, 1049-1169 (1989).
9. R. E. Lang, K. Herrman, and R. Dietz, *Life Sci*., **32**, No. 4, 399-406 (1983).
10. L. N. Maslov and Yu. B. Lishmanov, *Int. J. Cardiol*., **40**, No. 2, 89-94 (1993).
11. N. Pencheva, Jan Pospisek, L. Hauzerova, *et al*., *Br. J. Pharmacol*., **128**, 569-576 (1999).
12. G. E. Sander and T. D. Giles, *Biochem. Pharmacol*., **31**, 2699-2700 (1982).