7. R. Gay, S. Wool, M. Paquin, and G. Steven, Am. J. Physiol., **251**, H483 (1986).
8. E. A. Gorodetskaya, S. F. Dugin, O. S. Medvedev, and E. A. Allabergenova, J. Pharmacol. Meth., **24**, 43 (1990).
9. M. A. Heymann, B. D. Payne, J. I. B. Hoffman, and A. M. Rudolf, Prog. Cardiovasc. Dis., **20**, 55 (1977).
10. S. Kubo, A. Nishioka, H. Nishimura, et al., J. Cardiovasc. Pharmacol., **7**, 753 (1985).
11. M. G. Nicholis, H. Ikram, M. A. Fitzpatrick, and I. G. Crozier, Eur. Heart J., **9**, Suppl., 77 (1988).
12. M. Packer, J. Cardiovasc. Pharmacol., **10**, Suppl. 7, S83 (1987).
13. A. J. G. Riegger, Cardiology, **76**, Suppl. 2, 42 (1989).

ROLE OF CENTRAL AND PERIPHERAL MU- and DELTA-OPIATE RECEPTORS IN MECHANISMS OF THE ANTIARRHYTHMIC ACTION OF ENKEPHALINS

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The writers showed previously that preliminary injection of the synthetic Leu-enkephalin analog D-Ala^2, Leu^5, Arg^6-enkephalin (dalargin) into experimental animals prevents the onset of ventricular fibrillation due to acute myocardial ischemia (AMI) [4]. Almost at the same time identical results were obtained in Professor F. Z. Meerson's laboratory [14]. Both dalargin and its enzymic hydrolysis products can interact with mu-opiate receptors (OR), although their affinity for delta-OR is much greater [1].

It is thus difficult to give an unequivocal answer to the question of the role of either type of OR in the realization of the antiarrhythmic effect of enkephalins, more especially because data in the literature on this question are contradictory [2, 7, 8, 10, 11, 15].

The aim of this investigation was to study the contribution of mu- and delta-OR in the mechanism of the antiarrhythmic action of enkephalins.

EXPERIMENTAL METHOD

Experiments were carried out on 158 male rats weighing 200-250 g. A disturbance of the electrical stability of the myocardium was induced by occlusion of the left descending coronary artery [8]. Dalargin was injected into the femoral vein 10 min before coronary occlusion in a dose of 0.1 mg/kg which, as the writers showed previously, prevents arrhythmias and stress-induced myocardial damage [3, 4]. Morphine was injected intravenously 10 min before AMI in a dose of 1.5 mg/kg, which possesses antiarrhythmic activity [11]. Naloxone also was injected intravenously 5 min before injection of dalargin or 15 min before AMI, in a dose of 0.5 mg/kg to block mu-OR [6, 12], and in a dose of 1 mg/kg to block mu-, delta-, and kappa-OR [6, 12]. Dalargin, in a dose of 1 or 10 μg in 10 μl of 0.9 NaCl, was injected into the 4th cerebral ventricle at the rate of 2 μl/min, through a hollow needle implanted stereotaxically previously (5 days before the experiment), as was done

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TABLE 1. Effect of OR Agonists and Antagonists on VFT During AMI (M ± m)

Preparation	Dose, mg/kg	VFT, MA	n	Significance of differences
Control (AMI)	—	2,50±0,27	25	—
Dalargin	0,1	4,01±0,28	27	$p_1<0,001$
Dalargin	1	5,80±0,58	10	$p_1<0,001$
Naloxone + dalargin	0,5±0,1	4,16±0,63	12	$p_2<0,01$ $p_1<0,01$
Naloxone + dalargin	1±0,1	2,10±0,28	10	$p_1>0,05$ $p_2<0,001$
Morphine	1,5	3,64±0,35	10	$p_1<0,05$
Naloxone	0,5	2,90±0,40	9	$p_1>0,05$
Naloxone	1,0	1,25±0,13	8	$p_1<0,01$

Legend. n) number of animals; p_1) significant differences compared with control; p_2) significant differences compared with group of animals receiving dalargin in a dose of 0.1 mg/kg.

in studies of the central effects of enkephalins on the cardiovascular system [9]. The electrical ventricular fibrillation threshold (VFT) was determined with the help of an ES-50-1 cardiostimulator, triggered from the R wave of the ECG by application of single testing pulses with a duration of 2 msec and increasing current strength during the vulnerable phase of the cardiac cycle. Stimulation was carried out 5 min after coronary occlusion. The results were subjected to statistical analysis by Student's test.

EXPERIMENTAL RESULTS

Preliminary injection of dalargin in a dose of 0.1 mg/kg largely prevented the lowering of VFT caused by AMI (Table 1). Injection of dalargin in a dose of 1 mg/kg led to an even greater increase of VFT. Dalargin, as we know, is a mixed agonist of mu- and delta-OR [1].

A characteristic feature of activation of central mu-OR is bradycardia [5, 10]. Our investigations of intact animals showed that dalargin, in a dose of 0.1 mg/kg, does not affect the cardiac rhythm, but in a dose of 1 mg/kg it causes a transient decrease in heart rate (HR) at the 5th minute after intravenous injection of the peptide, from 348 ± 10 (n = 9) to 240 ± 15 beats/min (n = 9, $p < 0.001$). Consequently, the more effective action of the larger dose of dalargin on VFT may evidently be explained by activation of both types of OR simultaneously.

Injection of naloxone 5 min before dalargin, in a dose of 0.5 mg/kg, sufficient to block mu-OR [6, 12], did not abolish the effect of dalargin in VFT. Meanwhile, blocking peripheral delta- and kappa-OR by 1 mg/kg naloxone [6, 12] completely prevented any effect of dalargin on VFT (Table 1).

It can thus be concluded that an increase in electrical stability of the myocardium under the influence of dalargin is brought about mainly through activation of delta-OR, but, if the injected dose is increased, mu-OR may evidently also be involved in the process of raising VFT. It still remains unclear whether endogenous ligands of OR can participate in the increased resistance of the myocardium to arrhythmogenic influences.

As Table 1 shows, injection of the selective mu-OR antagonist morphine 10 min before coronary occlusion prevented the lowering of VFT, in agreement with data in the literature on the antiarrhythmic activity of opiates [8, 10, 11]. Injection of naloxone in a dose of 0.5 mg/kg did not affect VFT, but in a dose of 1 mg/kg naloxone significantly facilitated the lowering of VFT.

The results are evidence that activation of delta-OR, of which enkephalins are endogenous agonists [5], is an important factor in the prevention of arrhythmias induced by myocardial ischemia. With respect to endogenous agonists of mu-OR we did not observed any such effect. Meanwhile, a report has appeared in the literature that endogenous ligands of mu-OR play an important role in the prevention of arrhythmias [2]. Evidently this disparity can be explained by differences

in the techniques used to assess the antiarrhythmic activity of the drugs. For instance, the authors cited in [2] assessed the role of endogenous agonists of mu-OR on the basis of the effect of naloxone on the frequently of arrhythmias during the 15 min after coronary occlusion, whereas we assessed the antiarrhythmic activity on the basis of VFT 5 min after ligation of the coronary artery.

It must be pointed out that the authors of some studies, who used naloxone in doses above 1 mg/kg, found that this drug has an antiarrhythmic effect, probably linked with blocking of other types of OR, for we know that blocking of all types of OR can be produced only by the use of naloxone in a dose of 10 mg/kg [13], in which it exhibits maximal antiarrhythmic activity [15].

Another unsolved problem is which OR — peripheral or central — mediate the antiarrhythmic effects of enkephalins. To solve this problem we undertook a series of investigations into the effect of intraventricular injection of dalargin on the cardiac rhythm and VFT. The peptide was injected into the 4th ventricle, for we know that the antiarrhythmic and chronotropic effects of mu-antagonists are realized through activation of the vagus nerve nuclei, located in the medulla [5, 10, 11].

Infusion of dalargin into the 4th ventricle in a dose of 1 μg had no effect on the cardiac rhythm of intact animals and on VFT after coronary occlusion. An increase in its dose to 10 μg caused bradycardia in the intact animals and raised VFT from 1.96 ± 0.30 mA (n = 14) to 4.98 ± 0.29 mA (n = 13, $p < 0.001$) in animals with AMI, if the drug was injected 10 min before coronary occlusion.

The simplest calculation of the doses of dalargin injected per kilogram brain tissue (the weight of the brain in rats is about 1 g) indicates that the opiatergic effects observed following interventricular injection are not connected with activation of central delta-OR, for injection of 1 μg of the drug creates a concentration in nerve tissue of not less than 1 mg dalargin/kg. We have already stated that with this dosage of dalargin both mu and delta-OR are excited. However, after intraventricular injection opiatergic effects on VFT and HR did not appear. Consequently, the bradycardia and elevation of VFT which we found after intraventricular injection of dalargin can more logically be interpreted from the standpoint of stimulation of central mu-OR, more especially because the onset of bradycardia is a very characteristic feature of such stimulation [5, 10].

The results thus suggest that the antiarrhythmic effects of enkephalins in AMI are realized through activation of peripheral delta receptors and central mu-receptors.

LITERATURE CITED

1. N. V. Korobov, Neuropeptides: Their Role in Physiology and Pathology [in Russian], Tomsk (1985), pp. 73-74.
2. G. I. Kositskii, S. D. Mikhaiiova, S. L. Goroznan, and T. M. Semushkin, Byull. Éksp. Biol. Med., No. 5, 258 (1989).
3. Yu. B. Lishmanov, Byull. Éksp. Biol. Med., No. 9, 271 (1986).
4. Yu. B. Lishmanov, L. V. Maslova, T. V. Fedotova, and L. N. Maslov, Disturbance of Mechanisms of Regulation and Their Correction [in Russian], Vol. 2, Moscow (1989), p. 613.
5. P. V. Sergeev and N. L. Shimanovskii, Receptors of Physiologically Active Substances [in Russian], Moscow (1987).
6. A. I. Faden and T. P. Jacobus, Eur. J. Pharmacol., **91**, 321 (1983).
7. O. Fagbemie, I. Lepran, and J. R. Parrot, Brit. J. Pharmacol., **76**, 504 (1982).
8. O. Fagbemie, K. A. Kane, and I. Lepran, Brit. J. Pharmacol., **78**, 455 (1983).
9. W. Feldberg and E. Wei, J. Physiol. (London), **280**, 180 (1978).
10. E. Frey, Resuscitation, **3**, 105 (1974).
11. E. Frey, G. Avril, and E. Hartung, Cah. Anesth., **29**, No. 5, 591 (1981).
12. M. Laubie and H. Schmitt, Eur. J. Pharmacol., **71**, No. 4, 401 (1981).
13. C. Llorens-Cortes and J.-C. Schwartz, Eur. J. Pharmacol., **104**, 369 (1904).
14. F. Z. Meerson, M. G. Pshennikova, L. M. Belkina, et al., C. V. Wld. Res., **3**, 28 (1989).
15. T. M. Wong and A. Y. S. Lee, Clin. Exp. Pharmacol. Physiol., **12**, 379 (1985).