Antiarrhythmic Effect of Hypoxic Preconditioning Is Mediated by Activation of μ- and δ-Opioid Receptors

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny,* Vol. 125, No. 3, pp. 272-274, March, 1997
Original article submitted December 26, 1996

Adaptation of rats to repetitive hypoxia leads to a decrease in the severity and frequency of arrhythmias induced by epinephrine. Naloxone abolishes antiarrhythmic effect of adaptation. Activation of μ-and δ-opioid receptors is one of the important factors mediating antiarrhythmic effect of adaptation. Intravenous administration of acetorphan, an enkefalinase inhibitor, produces statistically significant antiarrhythmic effect in the control group. Thus, the antiarrhythmic effect of hypoxic adaptation results from activation of μ-and δ-opioid receptors due to increased level of endogenous enkefalins.

Key Words: *opioid receptors; opioid peptides; hypoxic adaptation; arrhythmia*

Hypoxic preconditioning protects the myocardium against electrical and contractile disfunction caused by experimental myocardial infarction [3]. However, the mechanisms of this effect are still unknown. Previously, we have shown that endogenous opioid system plays an important role in realization of antiarrhythmic effect of adaptation to short-lasting stress [2]. Moreover, it was found that the ligands of endogenous opioid receptors (OR) participate in the development of adaptational stability of organism to hypoxia [7]. Thus, it can be suggested that opioid system is involved in hypoxia-evoked protection of the myocardium against arrhythmogenous stimuli. This hypothesis requires experimental confirmation.

Our aim was to evaluate the role of endogenous μ-and δ-OR ligands in antiarrhythmic effect of hypoxic preconditioning.

METHODS

Wistar rats (body weight 150-200 g) were used. Adaptation to hypoxia was performed in an altitude chamber by gradual "lifting" of the animals to the height of 5000 m above the sea level for 6 h every day during 45 days. Such an adaptation protocol prevented the development of cardiac contractile disfunction in experimental coronary occlusion [3]. Arrhythmias were provoked by intravenously injected epinephrine (100 μg/kg) under weak ether anesthesia. The ECG was recorded during 5 min after injection, and the frequency of ventricular arrhythmias (paroxysmal tachycardia, exstrasystole, and fibrillation) was assessed for each experimental group.

To evaluate the participation of endogenous opioid system in antiarrhythmic effects of hypoxic adaptation we pretreated the rats with the following OR antagonists or enkephalinase inhibitors:

1) naloxone, a nonspecific blocker of OR (Sigma), used in a low concentration of 0.2 mg/kg, which affects only μ-receptors [4,7], and in a high concentration of 2 mg/kg, which inactivates almost any type of OR;

2) ICI 174,864 — (N,N-diallyl-Tyr-Aib-Aib-Phe-Leu-OH)[N,N-dial-lyl-Tyr1,Aib2,3] Leu-enkephalin ("Chiron Mimotopes Peptide Systems"), a selective antagonist of δ-OR (2.5 mg/kg) [6].

3) CTAP — (H-D-Phe-Cys-Tyr-D-Trp-Arg-Thr-Pen-Thr-NH_2) (Chiron), a high-specific blocker of peripheral μ-OR [9,10] (1.0 mg/kg). Special experiments showed that this concentration was sufficient to abolish antiarrhythmic effect of peripheral μ-OR agonists.

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0007-4888/98/0003-0239$20.00

Table 1. Effect of OR Antagonists on Epinephrine-Induced Arrhythmias in Hypoxia-Adapted Rats

Groups	Ventricular extrasystole						Ventricular tachycardia		Ventricular fibrillation	
	WVE		SVE		MVE					
	n	%	*n*	%	*n*	%	*n*	%	*n*	%
Control (*n*=25)	0	0	7	28	18	72	8	32	7	28
Adaptation (*n*=15)	12	80*	2	13	1	7*	0	0*	0	0*
Adaptation+Naloxone, 2 mg/kg (*n*=15)	2	13+	4	26	9	60+	0	0*	0	0*
Adaptation+Naloxone, 0.2 mg/kg (*n*=15)	0	0+	1	7	14	93+	0	0*	1	7
Adaptation+ICI 174,864 (*n*=16)	1	6+	5	13	10	62+	0	0*	1	6
Adaptation+CTAP (*n*=15)	0	0+	1	7	14	93+	0	0*	2	13

Notes: *$p<0.0025$ versus control; +$p<0.001$ versus group of adapted animals. WVE — without ventricular extrasystoles, SWE — single ventricular extrasystoles, MVE — multiple ventricular extrasystoles.

4) acetorphan [(R,S)CH_3-COS-CH_2-CH($CH_{2\phi}$) CONH-CH_2-$CO_2CH_{2\phi}$], an enkephalinase inhibitor [5] kindly given by Prof. J.-C. Schwarz (Centre Paul Broca de l'INSERM, France) (10.0 mg/kg).

Both adapted and nonadapted animals injected intravenously with 0.9% NaCl (0.1 ml/100 mg) were used as controls. All injections were made 15 min before induction of arrhythmias. Statistical significance of the intergroup differences was estimated by the χ^2 test.

RESULTS

Hypoxic adaptation increased stability of rat hearts against arrhythmogenous action of epinephrine (Table 1). After adaptation, the number of animals without cardiac rhythm disturbances significantly increased by 6 times compared with the control, and malignant arrhythmias (ventricular tachycardia and fibrillation) were not observed.

The μ- and δ-OR blocker naloxone (2 mg/kg) partially attenuated antiarrhythmic effect by decreasing the number of rats without arrhythmia, but did not affect the frequency of malignant arrhythmias (ventricular tachycardia and fibrillation). This data argue in favor of participation of μ- and δ-OR in the development of antiarrhythmic effect of hypoxic adaptation.

In adapted animals, injection of the specific peripheral μ-OR inhibitor CTAP significantly increased the percentage of cardiac rhythm disturbances and fatal arrhythmias (ventricular fibrillation). This indicates that the antiarrhythmic effect induced by hypoxic adaptation is mediated via peripheral μ-OR.

Injection of the specific δ-OR blocker ICI 174,864 under the same conditions led to a significant decrease in the number of rats resistant to arrhythmogenic action of epinephrine and in a 10-fold increase in the frequency of extrasystoles. This can be considered as an indirect evidence for the importance of δ-OR activation in adaptation processes.

Our previous study showed that adaptation increases enkephalin levels in the brain, plasma, and myocardium [1]. This prompted us to study the influence of artificially increased levels of endogenous enkephalins on the frequency and severity of epinephrine-induced arrhythmias.

Table 2. Effects of Acetorphane and Acetorphane Combined With OR Inhibitors on Epinephrine-induced Arrhythmias

Groups	Ventricular extrasystole						Ventricular tachycardia		Ventricular fibrillation	
	WVE		SVE		MVE					
	n	%	*n*	%	*n*	%	*n*	%	*n*	%
Ventricular fibrillationControl (*n*=25)	3	12	5	20	16	64	4	16	4	16
Acetorphane (*n*=15)	11**	73	3	20	1+	6	0	0	1	6
Acetorphane+Naloxone, 0.2 mg/kg (*n*=15)	4**	27	6	40	5	33	0	0	0	0
Acetorphane+ICI 174,864 (*n*=15)	0***	0	4	27	11***	73	1	7	0	0
Acetorphane+CTAP (*n*=15)	5**	33	10**	67	0**	0	0	0	0	0

Notes: *$p<0.01$, **$p<0.025$, ***$p<0.001$ versus group injected by acetorphane; +$p<0.05$, **$p<0.001$ *vs.* control group.

We showed that acetorphane given in concentration sufficient for suppression of enkephalinase activity *in vivo* [5] significantly increases heart resistance to arrhythmogenic concentrations of epinephrine: the number of animals without cardiac rhythm disturbances was greater than that in the control group (Table 2). Thus, our hypothesis that enkephalins participate in the enhancement of cardiac electrical stability was substantiated experimentally. It is known that enkephalins are nonselective endogenous agonists of μ- and δ-OR. Therefore, we carried out experiments for elucidating the role of each type of μ- and δ-OR using specific blockers. Blockade of each type of these OR led to reduction of antiarrhythmic effect of acetorphane (see Table 2). These data also confirm the hypothesis on the participation of endogenous μ- and δ-OR ligands in the adaptation process.

Thus, our results show that activation of μ- and δ-OR due to an increase in the levels in endogenous enkephalins induced by hypoxic preconditioning is one of the important factors mediating the antiarrhythmic effect of adaptation.

We are thankful to P. Hillary, Ph.D. (NIDA,US) and Prof. J.-C. Schwarz (Centre Paul Broca de l'INSERM, France) for the help and support of this study.

REFERENCES

1. Y. B. Lishmanov, L. N. Maslov, Opiate neuropeptides, stress, and adaptational heart protection, Tomsk (1994).
2. Y. B. Lishmanov, E. V. Uskina, L. N. Maslov, A. V. Krilatov, *Byull. Eksp. Biol.*, **122**, No. 9, 276-278 (1996).
3. F. Z. Meerson, Adaptation, Stress and Prophylactic, M (1981).
4. H. D. Allescher, S. Ahmad, E. E. Daniel, et al., *Am. J. Physiol.*, **255**, No. 3, Pt. 1, G352-G360 (1988).
5. P. R. Burnard, F. Noble, V. Dauge, et al., *Pharmacol. Rev.*, **45**, No. 1, 87-146 (1993)
6. R. Cotton, M. G. Giles, L. Miller, et al., *Eur. J. Pharmacol.*, **97**, 331-332 (1984).
7. K. P. Mayfield, L. G. D'Alecy, *Brain Res.*, **582**, 226-231 (1992).
8. M. J. Millan, F. C. Colpaert, *Neuroscience*, **42**, No. 2, 541-553 (1991).
9. J. T. Pelton, W. Kazmierski, K. Gulya, et al., *J. Med. Chem.*, **29**, 2370-2375 (1986).
10. J. E. Shook, J. T. Pelton, W. Kazmierski, et al., *NIDA Res. Monogr.*, **76**, 295-301 (1986).