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Mechanism of the Antiarrhythmic Effect of Agonists and Antagonists of Opioid Receptors

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Infranodal arrhythmia is one of the most crucial problems in modern cardiology. Recent investigations have demonstrated the antiarrhythmic activity of enkephalins [4,6], but only one synthetic analog of enkephalins - dalargin - was used in these studies. We have not found any published data on the antiarrhythmic properties of other opioid peptides (OP). Moreover, there is a prevailing opinion that the endogenous OP are arrhythmogenic agents, because naloxone, an opioid receptor (OR) antagonist, has an antiarrhythmic activity [9,17].

It thus seemed interesting to study the antiarrhythmic effect of OR agonists and antagonists and to reveal its possible mechanism.

MATERIALS AND METHODS

Experiments were carried out on 508 male Wistar rats weighing 150-200 g under ether anesthesia.

Arrhythmia was simulated by i.v. epinephrine injection in a dose of 90 μg/kg [10]. The electrocardiogram was recorded in the standard lead II 5 min after injection.

OR ligands were administered 15 min before and 6 h after epinephrine injection. D-Ala2-Leu5-Arg6-enkephalin (dalargin, Research Institute of Experimental Cardiology of the Russian Academy of Medical Sciences), Des-leucyl5-Ala 2[β-4-nitrophenyl]-α-alaninamide-4 enkephalin (tetrapeptide, Research Institute of Experimental Cardiology), D-Ala2-D-Leu5-enkephalin (DADLE, Vector Scientific-Conglomerate), Leu-enkephalin (Serva, Germany), and D-Met2-Pro5-enkephalinamide (enkephalinamide, Institute for Drug Research, Hungary) were used. The peptides were dissolved in 0.9% NaCl ex tempore. We demonstrated previously [4] that dalargin in a dose of 0.1 mg/kg expresses a high antiarrhythmic activity, and therefore the OP were injected in a dose of 0.1 mg/kg. An antiarrhythmic dose of morphine was 1.5 mg/kg [10]. Naloxone was injected in a dose of 0.5 mg/kg, which produced a blocade of the μ-OR [12], and in a dose of 2 mg/kg, enough to block all types of OR and to prevent arrhythmia [9,12,17]. Control animals were treated with epinephrine and NaCl instead of opioids. To obtain a complete inhibition of prostaglandin synthesis [8], the animals were treated with indomethacin together with

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TABLE 1. Effect of OR Ligands on Frequency of Occurrence of Infranodal Extrasystole (IE) ($M \pm m$)

Index	15 min after administration of the drugs			6h after administration of the drugs		
	n	without IE	with IE	n	without IE	with IE
Control	20	1	19	—	—	—
Dalargin	15	2	13	16	9*	7*
Morphine	17	11*	6*	16	10*	6*
Tetrapeptide	15	3	13	16	7	7*
Enkephalinamide	10	9*	1*	10	8*	1*
Naloxone, 0.5 mg/kg	15	6*	9*	15	6	9
Naloxone, 2 mg/kg	15	9*	6*	15	12*	3*
Leu – enkephalin	15	2	13	15	10*	5*
DADLE	15	9*	6*	15	10*	5*

Note. n is the number of experimental animals. Asterisk: $p<0.05$ as related to the control (epinephrine administration). Enkephalins were injected in a dose of 0.1 mg/kg.

the OR ligands (6 h before epinephrine administration) in a dose of 10 mg/kg in a 5% alcohol solution prepared using 0.9% NaCl ex tempore. Actinomycin D was injected in a dose of 0.25 mg/kg simultaneously with the OR ligands 6 h before epinephrine to cause a total block of protein synthesis [14]. Atropine was administered in a dose of 1 mg/kg 15 min prior to opioid injection to abolish the parasympathetic effects. All drugs were injected in the femoral vein.

The results were processed statistically using the χ^2-test [1].

RESULTS

It was revealed (Table 1) that the antiarrhythmic effect of enkephalins was most pronounced 6 h after injection. However, enkephalinamide and DADLE (synthetic analogs of enkephalins) prevented the epinephrine-induced arrhythmia as early as 15 min and also 6 hours after administration. It is known that the serum "lifetime" of enkephalin synthetic analogs is no more then 30 min *in vitro* [2], and thus these findings suggest that enkephalins have a delayed effect.

Thus, Δ-OR agonists (enkephalins) prevented the occurrence of epinephrine-induced arrhythmia. According to published data, the synthetic analogs of enkephalins cross the blood-brain barrier when injected systemically in doses exceeding 0.5 mg/kg [7], and we used a dose of 0.1 mg/kg. It may therefore be assumed that the antiarrhythmic effect of the enkephalins probably relates to an activation of the peripheral OR. It is to be noted, however, that the central μ-OR may take part in arrhythmogenesis, because the selective μ-agonist morphine and nonselective OR blocker naloxone exhibit an antiarrhythmic activity in a dose of 2 mg/kg (Table 1).

These effects of naloxone and morphine are not easily interpreted. There are two subpopulations of μ-OR: the μ_1-OR, characterized by a high affinity for morphine and a very low affinity for naloxone so that the latter cannot block μ_1-OR even in a dose of 50 mg/kg, and the μ_2-OR with a high affinity both for morphine and for naloxone, as reported by the Pasternak research group [16]. These scientists showed that naloxone, when used in low doses, blocks precisely the μ_2-OR [16]. It may thus be logically assumed that the antiarrhythmic effect of morphine relates to an activation of the μ_1-OR, while the effect of naloxone is associated with a blockade of the μ_2-OR.

The findings enable us to assume that the mechanisms of the "early" and "late" antiarrhythmic effects produced by the OR ligands may differ significantly from each other. Enkephalins probably "trigger" the biosynthesis of certain biologically active messengers that direct the "late" antiarrhythmic effect of the OR. These messengers might be thought to be be prostanoids and proteins because we showed previously that dalargin activates protein and prostanoid biosynthesis in the myocardium [3,5]. But the experiments involving pretreatment with actinomycin D (an inhibitor of DNA-dependent RNA polymerase) or with indomethacin (an inhibitor of cyclooxygenase) showed that these drugs do not affect the "late" antiarrhythmic effects of the OR ligands.

Thus, the results obtained demonstrate that neither prostanoids nor proteins can be the messengers for the "delayed" antiarrhythmic effects of the OR agonists and antagonists.

An alternative mechanism of the antiarrhythmic opioid action could be a change in the state of the autonomic nervous system.

The recent consensus is that activation of the sympathetic-adrenal system (SAS) promotes the

initiation of arrhythmia [13], whereas an enhanced tonicity of the vagus nerve, on the contrary, greatly increases the electrical stability of the heart [15]. We demonstrated previously that dalargin lowers the cAMP content and elevates the cGMP level in the rat myocardium [3], testifying to a reduced activity of SAS and increased vagal tone.

It is reported [10] that a certain role in the antiarrhythmic effects of the μ-agonists is played by activation of the parasympathetic influence on the heart.

We showed that atropine injected in animals does not abolish the "early" and "late" antiarrhythmic effects of opioids. Hence, the parasympathetic nervous system does not play any essential role in realizing the antiarrhythmic effects of the OR agonists. It may thus be assumed that enkephalins prevent the epinephrine-induced arrhythmia by limiting the adrenergic influence on the myocardium, for example, by inhibition of adenylate cyclase [11].

Thus, the findings indicate the leading role of the antiadrenergic properties of the OR ligands in the realization of their antiarrhythmic effects.

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