Contribution of μ- and δ-Opiate Receptors into Vagotropic Effect of Met-Enkephalin

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In anesthetized cats met-enkephalin affects the initial cardiac rhythm and synchronizing component of vagal chronotropic control via excitation of δ-opiate receptors and modulates tonic inhibitory vagal control via activation of both δ- and μ-opiate receptors.

Key Words: *vagus; opioids, cardiac rhythm*

Activation of opioid system inhibits vagal influences on the heart. Moderation of chronotropic effect (CE) of the vagus nerve (VN) was revealed in experiments with morphine [10,11]. Later a similar effect was found with endogenous opioids, the enkephalins [3,4,6,12]. The suppressing effect of enkephalins is manifested only in the respect of tonic inhibitory vagal control. By contrast, enkephalins paradoxically increase the synchronizing component of parasympathetic CE [3,4]. Our aim was to study the receptor mechanism that mediates these effects of opioids. Met-enkephalin is known as a versatile agonist with affinity for μ-, and δ-opioid receptors (OR) [1,9]. In this respect it is intriguing to study the dynamics of vagal CE under individual activation or, on the contrary, under individual inhibition of various types of OR. We used the ligands that are highly selective for μ- and δ-OR.

MATERIALS AND METHODS

Experiments were carried out on 50 cats (body weight 3-4 kg) narcotized intraperitoneally with a Chloralose-Nembutal mixture (75 and 15 mg/kg, respectively) and artificially ventilated. The peripheral stump of the right VN was stimulated with the trains of 6 rectangular voltage pulses. Duration and frequency of the pulses in a train were 2 msec and 40 Hz; the amplitude was 5-6 threshold values. Intracardiac ECG was recorded by means of a unipolar probe inserted through the femoral vein into the right atrium; the *P* wave of the ECG triggered the recording of the heart cycle intervalogram. The tested substances were ME (20 μg/kg), D-Ser^2-Leu^5-Thr^6-enkephalin (DSLET, selective agonist of δ-OR, 20 μg/kg), morphiceptin (selective agonist of μ-OR, NH_2-Tyr-Pro-Phe-Pro-$CONH_2$, 20 mg/kg), naloxonazine (agonist to μ-OR, 0.3 mg/kg), and naltrindole (agonist of δ-OR, 0.3 mg/kg). All substances were infused intravenously in streams of physiological saline (0.3 ml). The data were statistically analyzed using the method of direct differences [2].

RESULTS

Injection of ME slowed down heart rate (HR) in narcotized cats and increased the duration of the cardiac cycle from 318.4±8.5 to 347.5±10.2 msec, i.e., by 9.1% ($p<0.02$). The latency and duration of its effect was 12.6±4.4 and 347.3±10.6 sec, respectively. The effect of ME was not modified by blockade of μ-OR with naloxonazine, and was completely prevented by intravenous injection of the δ-OR antagonist naltrindole or the M-cholinolytic atropine (0.1 mg/kg). Excitation of μ-OR by morphiceptin did not affect the duration of cardiac cycle which was increased by the selective agonist of δ-OR

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DSLET [9]. This increase was 13.1% in comparison with the initial value, the latency and duration being 20.8±5.4 sec and 20-30 min, respectively. A longer effect of DSLET in comparison with that of ME is probably due to enzymatic resistance of this peptide caused by incorporation of D-Ser^2 and Thr^6 in its structure, which enhances the peptide resistance to enkephalinases [1,9].

Stimulation of VN caused bradycardia that was accompanied by synchronization of heart beats to the volley rhythm of vagal stimulation. Within the limits of a certain range each burst of pulses was accompanied by a single heart beat. The initial HR was 190.4±7.6 beat/min, the upper and lower boundaries of synchronization range (SR) were, respectively, 111.7±7.8 and 92.1±6.9. The width of SR (19.6±1.8 beat/min) reflects the degree of synchronizing component of vagal CE, while the difference between the initial HR and that at the upper limit of SR (78.7± 6.1 beat/mean) determines the value of tonic component. The sum of this components (98.3±8.2) corresponds to the total value of vagal CE.

Administration of ME decreased vagal CE and its tonic component by, respectively, 17.2 and 20.6% relative to the initial values. By contrast, ME augmented the width of vagocardiac SR by 15.5%. This dynamics corresponds to the specificity of the vagotropic effect of ME [3,4]. Similar changes in the structure of vagal CE were produced by DSLET (Table 1). In addition, it decreased the degree of vagal CE and diminished the inhibitory tonic component by 16.4% and 23.6%, respectively. DSLET increased the synchronizing vagal component by 12.8%, the increase being 24% 15 min after its administration. Morphiceptin diminished the total value of vagal CE and its tonic component by 8.0 and 11.5%, respectively, but did not affect the width of vagocardiac SR.

Naloxonazine, a selective antagonist of μ-OR, [8], had no individual effect on vagal control of HR and did not affect the dynamics of ME-induced vagotropic effect (Fig. 1). The antagonist of δ-OR naltrindole [13] decreased the width of vagocardiac SR by 14.1%. The synchronizing vagal component remained diminished after subsequent infusion of ME. Thus, the potentiating effect of ME on this component was eliminated by blockade of δ-OR. Naltrindole did not prevent the development of inhibitory effect of ME on vagal CE and its tonic component (Fig. 1). These indices decreased by 13.4 and 12.5%, respectively.

There is evidence that the affinity of DSLET for δ-OR is 620-fold as high as that for μ-OR [9]. In other words, DSLET is a highly selective agonists for δ-OR. Indeed, the affinity of ME for δ-OR is only 15-fold higher than that for μ-OR [9]. Another selective opioid agonist, morphiceptin, is a tetrapeptide, an amidated fragment of β-casomorphin which is a natural opioid isolated from milk extracts [5]. The affinity of morphiceptin for μ-OR is about 1000-fold as high as that for δ-OR [7]. The use of these peptides and of the specific opioid antagonists naloxonazine and naltrindole, made it possible to evaluate the contribution of μ- and δ-OR to the cardiotropic effects of ME. Stimulation of δ-OR plays the key role in the effect of ME on HR, since a decrease in HR caused by ME is blocked by the δ-OR antagonist naltrindole and restored by DSLET (Table 1). By contrast, the μ-OR agonist morphiceptin did not produce this effect. These findings can be explained by different density of OR distribution in various types of cardiac structures, which have mostly δ-type OR [1].

Both μ- and δ-OR participate in the modulation of tonic inhibitory vagal influence, and in both cases their activation results in inhibitory vagal effect which was observed both with DSLET and morphiceptin (Table 1). The role of μ-opiate reception was also demonstrated by infusion of the δ-OR antagonist naltrindole that did not completely suppress the ME-induced decrease in the tonic inhibitory vagal effect. These findings support the observation that stimulation of presynaptic OR inhibits vagal influence on isolated rabbit heart [14]. At the same

TABLE 1. Dynamics of Vagal Heart Regulation under the Effect of Opioids

Substances	Initial GR	Vagal component		Vagal CE
		synchronizing	tonic	
ME	—	+	—	—
DSLET	—	+	—	—
Morphiceptin	0	0	—	—
ME+naloxonazine	—	+	—	—
ME+naltrindole	0	0	—	—

Note. (+) indicates increase and (-) decrease of the corresponding index; *0*) no effect.

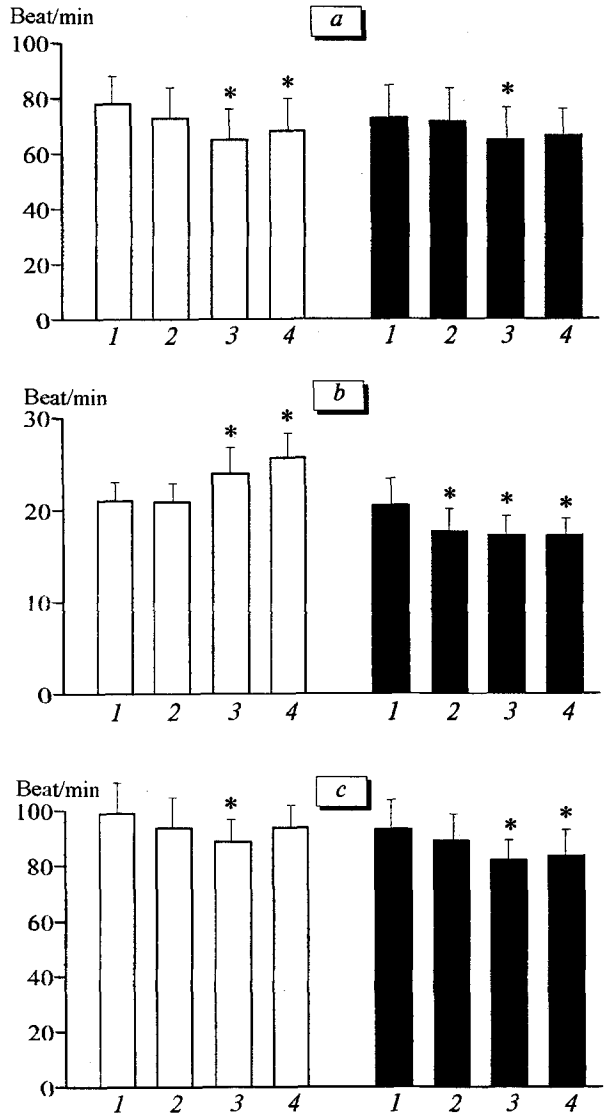

Fig. 1. Dynamics of vagotropic effect of met-enkephalin (ME) against the background blockade of various opiate receptors. Shown are (*a*) the dynamics of tonic effect, (*b*) dynamics of synchronizing vagal component, and (*c*) vagal chronotropic effect caused by ME against the background of the μ-opioid antagonist naloxonazine (open bars) or δ-opioid antagonist naltrindole (solid bars). *1*) initial value; *2*) after infusion of opioid antagonist; *3*) ME; *4*) 15 min postinfusion of ME. $p<0.05$: in comparison with the initial value.

time, the vagocardiac synchronizing effect produced by volley stimulation of VN is not affected by activation or blockade of μ-OR, but it is exclusively modulated by opioids via the δ-OR route. Blockade of δ-OR by naltrindole decreased SR, while their activation by DSLET or ME produced an opposite effect. Thus, activation of δ-OR may lead to complex rearrangements of the functional structure of vagal CE which includes not only suppression of the tonic inhibitory influence, but also simultaneous potentiation of the vagal synchronizing effect.

Directionality of vagotropic effect caused by stimulation of various types of OR also depends on the initial state of parasympathetic control of the heart. It can be suggested that the tonic release of acetylcholine from vagal terminals is augmented during activation of presynaptic δ-OR. This is confirmed by the ME-induced decrease in HR, which is atropine-dependent and is prevented by the δ-OR antagonist naltrindole. The release of acetylcholine caused by vagal stimulation is inhibited during activation of δ-OR, which explains the moderation of vagal CE by DSLET or ME. Activation of μ-OR does not modify the vagal control in the initial conditions, but moderates enhanced vagal influence. These findings attest to potential efficiency of opioids when cardiac regulation is disturbed by either deficiency or extra activation of the cholinergic chronotropic influences.

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