Specificity of Vagotropic Peptide Action under Subtotal Blockade of Cardiac M-Cholinoreceptors

O. E. Osadchii and V. M. Pokrovskii

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In experiments on anesthetized cats modulation of the components of vagal chronotropic effects by somatostatin and Met-enkephalin is studied under complete block of M-cholinoreceptors caused by administration of the M_2-cholinoblocker Imperialine or the subthreshold doses of the non-selective M-cholinolytic agent methacine. A differential character of vagotropic effect of peptides is revealed, which is manifested in restoration of the synchronizing vagal component that was decreased by cholinolytics, while a similar effect on the inhibitory tonic vagal influence was absent.

Key Words: *vagal chronotropic effect; somatostatin; Met-enkephalin; M-cholinoreceptors*

Stimulation of the vagus nerve (VN) by a short train of electric pulses causes controlled bradycardia associated with synchronization of the vagal and cardiac rhythms [5]. The nature of this phenomenon cannot be explained entirely by the classical cholinergic mechanisms: the regulator peptides play a certain role in it [6,15]. Specifically, it is shown that both somatostatin (SS) and Met-enkephalin (ME) increase the frequency range in which vagal stimulation controls cardiac rhythm [7,15]. A possible mechanism of this peptide action can be sensitization of cardiac M-cholinoreceptors to the synchronizing component of the vagal chronotropic influence. Therefore, the aim of this work was to study the vagotropic action of SS and ME under subtotal blockage of M-cholinoreceptors caused by administration of cholinolytics.

MATERIALS AND METHODS

Experiments were carried out on 24 cats weighing 2.5-3.5 kg narcotized intraperitoneally with Chloralose—Nembutal mixture (75 and 15 mg/kg, respectively) and artificially ventilated. The peripheral end of the right VN was stimulated with trains of 9 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. Amplified ECG was recorded by means of a unipolar probe inserted through the femoral vein into the right atrium. The chemical substances dissolved in 0.5 ml physiological saline were administered intravenously in streams. The doses were $3.0\text{-}4.0\times10^{-4}$ mg/kg (methacine), 0.01 mg/kg (Imperialine), and 50 μg (SS and ME, Sigma). Methacinum concentration was chosen in accordance to its dose-dependent vagotropic effect [8]. The interval between administration of cholinolytics and peptides was 10-12 min. In the control experiments carried out against the background of cholinolytic action, peptide solution was replaced by an equivalent volume of physiological saline. The data were statistically analyzed using the method of direct differences [4].

RESULTS

The initial duration of the cardiac cycle in cats was 312.5±10.4 msec. The range of controlled bradycardia was found under conditions of vagal stimulation, in which every train of vagal pulses was accompanied by a single heart beat. Variations in the inter-

Department of Normal Physiology Kuban State Medical Academy, Krasnodar

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val between vagal trains resulted in equally manifested change in duration of cardiac cycle, which made it possible to tune its value precisely within the range from 591.4.±12.3 to 750.0±15.6 msec (the upper and lower boundaries of the synchronization range). The width of this range corresponded to the degree of vagal synchronizing component, while the difference between the initial duration of cardiac cycle and the upper boundary of the synchronizing range corresponded to the level of tonic vagal component. The sum of both components corresponded to the total value of VN chronotropic effect (Table 1).

Methacine in a dose of 0.01 mg/kg completely blocked vagal influence on the heart. Smaller doses of this substance ($3.0\text{-}4.0\times10^{-4}$ mg/kg, $n=12$) decreased vagal chronotropic effect by 35%, which was accompanied by differential variation of its components. The value of synchronizing component decreased by 79%. At the same time, there was no significant decrease in the tonic inhibitory vagal influence. Administration of SS partially restored the synchronizing component (Table 1). The width of the controlled bradycardia range increased under the effect of the peptide up to 61.7±10.1 msec ($p<0.05$). It potentiated vagal chronotropic effect by 14.5%. A similar potentiation of synchronizing influence was observed after administration of ME (Fig. 1). In this case the increment was 33%, while 15 min after ME administration, the synchronization effect increased by additional 20.6%. This phenomenon conforms to the delayed character of the potentiation effect of this peptide on the controlled vagal bradycardia [7]. By contrast, the value of inhibitory tonic influence of VN decreased by 18% under the action of ME. Another feature of this peptide was an increase in the initial duration of cardiac cycle. Its value were 337.5±12.6 and 367.9±12.3 prior to and after administration of ME, respectively ($p<0.05$).

At present, at least 5 various subtypes of M-cholinoreceptors are known, which differ by their properties [16]; specifically, they have different sensitivity to various concentrations of cholinotropic substances [1]. One can suppose that selective inhibition of synchronizing vagal component results from blockade by methacine of a certain type of M-cholinoreceptors that are capable to interact with an antagonist even when the preparation is administered in the threshold dose. Taking into account the fact that cardiac cholinoreceptors are predominantly of the M_2 type [11], we studied vagotropic effect of the peptides against the background of the selective M_2-cholinolytic Imperialine [3]. The direction of vagotropic effect of this substance completely corresponded to the action of the threshold doses of Methacinum. Imperialine suppressed vagal chronotropic effect due to inhibition of its synchronizing component: numerically this inhibition was, respectively, by 24.9 and 52%. The value of inhibitory tonic effect did not change (Table 1). As in the experiments with methacine, administration of the peptides against the background of Imperialine caused an increase in the diminished value of the vagal synchronization effect. After administration of SS it was 48%; ME increased this index by 33.3% followed by subsequent increment of the potentiation effect by additional 24.7% 15 min postinjection of the peptide. The effect of ME on the duration of cardiac cycle and on the vagal tonic component was similar to the action of this peptide against the background of methacine.

The major result of this work is the revealing of the ability of SS and ME to restore vagal synchronizing component which was decreased by M-cholinolytics and which provides the control over cardiac rhythm under the volley stimulation of VN. Modulating effects of the peptides are usually considered on the basis of their presynaptic effect manifested in the change of the amount of transmitter released from the nerve terminals. However, this approach is probably of little practical use in analysis of the peptide effect on the dynamics of the vagal synchronizing component, because its value only slightly

TABLE 1. The Effects of Methacine, Imperialine, and SS on the Components of Vagal Chronotropic Action (msec, *M±m*)

Test substance	Initial duration of the cardiac cycle	Synchronization range boundaries		Component		Vagal chronotropic effec
		upper	lower	synchronizing	tonic	
Control data	312.5±10.4	591.4±12.3	750.0±15.6	158.6±13.4	278.9±14.5	437.5±18.4
Methacinum	315.0±11.2	566.1±13.1	599.4±13.4	33.3±9.9*	251.1±14.2	284.4±17.8*
Methacinum+SS	310.2±11.4	574.0±12.7	635.7±15.1	61.7±10.1**	263.8±13.9	325.5±16.6**
Imperialine	329.2±10.7	570.0±13.2	646.0±15.9	76.0±10.4*	240.8±14.1	316.8±17.2*
Imperialine+SS	315.0±10.8	571.3±13.4	683.8±14.8	112.5±10.6**	256.3±14.5	368.8±16.1**

Note. *$p<0.05$ relative to initial (control) data; **$p<0.05$ relative to the corresponding index against the background of cholinolytic action.

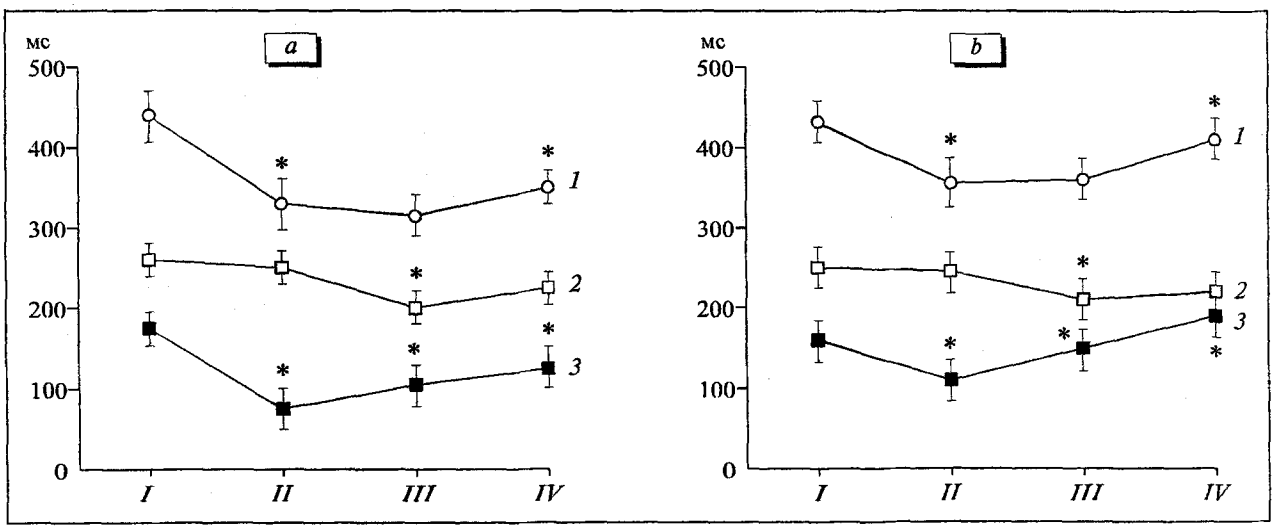

Fig. 1. The effect of Met-enkephalin on the parameters of the vagal chronotropic effect under subtotal blockage of cardiac M-cholinoreceptors after administration of (*a*) Methacinum or (*b*) Imperialine. *1*) vagal chronotropic effect; *2*) tonic component; *3*) synchronizing component. *I*) initial indices; *II*) after administration of (*a*) methacine or (*b*) Imperialine; *III*) after administration of Met-enkephalin; *IV*) 15 min after administration of Met-enkephalin. *$p<0.05$ in comparison with the preceding index.

depends on changes in the efficient concentration of acetylcholine [6]. The effect of some peptides at the postsynaptic level is similar to the effects observed during stimulation of VN. For example, it was shown that administration of SS leads to deceleration of cardiac rhythm, to negative dromotropic effect, and to a decrease in myocardial contractility [10,12,14]. It is supposed that during complete blockade of M-cholinoreceptors the inhibitory influence of VN on the heart is provided by the release of SS, which is stored in vagal terminals together with acetylcholine [12]. Similar to electrophysiological effect of acetylcholine, the effect of SS at the cellular level is manifested as increased calcium current [10,14]. Being M-cholinoreceptors, the receptors to SS are coupled to adenylate cyclase via inhibitory G proteins. In both cases stimulation of the receptors decreases intracellular cAMP content [11]. Taken together, these functional features are also characteristic of δ-opiate receptors through which the cardiotropic effects of enkephalins are predominantly mediated [2,9,13]. Such a parallelism could provide a basis for mutual potentiation of the effects of cholinergic and peptidergic regulatory systems, a manifestation of which is sensitization of cardiac M_2-cholinoreceptors to vagal synchronizing influence. On the other hand, to explain our findings, one should take into account the fact that both allosteric configuration of M-cholinoreceptors and their affinity for acetylcholine vary as a result of interaction of the peptides with their receptor. Examples of such interconnections between the receptor action of various hormonal regulators are available in the literature [2].

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