Dynamics of Vagal Chronotropic Effects during Blockade of Different Types of Muscarinic Cholinergic Receptors

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Different changes in vagal chronotropic effects were observed in cats under blockade of various types of muscarinic cholinergic receptors. Burst stimulation of the vagus nerve caused synchronization of the vagal and cardiac rhythms, which was potentiated by M_1 and M_3 receptor antagonists and suppressed by the M_2 blocker gallamine. The components of the vagal chronotropic effect showed different sensitivity to selective M-cholinolytics.

Key Words: *muscarini cholinergic receptors; vagal chronotropic effect*

Heterogenous population of muscarinic cholinergic receptors includes at least 5 subtypes which are distinguished by their structure, location, and functional properties [7]. In the myocardium, M_2 receptors are the predominant type of receptors. They mediate the inhibitory effects of the vagus nerve (VN) stimulation. However, there is evidence that other types of cholinergic receptors are also implicated in the regulation of cardiac activity. For instance in rats and guinea pigs, the intracardial neurons contain RNAs encoding four types of muscarinic receptors (M_1-M_4) [9]. Receptors of different types may play different functional roles. For instance, in dogs M_1 receptor blockade significantly weakened the vagal chronotropic effect (VCE), but did not affect the vagal influence on the myocardial conductivity and contractility [5,12].

These findings can be used in the analysis of VCE mechanisms. Along with the inhibitory tonic component, the chronotropic effect contains a synchronizing component that controls cardiac rhythm under conditions of VN stimulation [13]. These components are probably associated with different muscarinic receptors. In this study we checked up this hypothesis.

MATERIALS AND METHODS

Experiments were carried out on 27 cats weighing 3-4 kg anesthetized with intraperitoneal Chloralose-Nembutal mixture (75:15 mg/kg) and artificially ventilated. The peripheral end of the right VN was stimulated with short 40 Hz trains of 3, 6, or 9 rectangular pulses of 0.2 msec duration and 5-6 thresholds' intensity. Amplified ECG was recorded by a unipolar probe inserted through the femoral vein into the right atrium. The following selective M-receptor antagonists were used: pirenzepine (M_1), gallamine (M_2), and 4-diphenylacetoxy-N-(2-chlorethyl)-piperidine (4-DAMP, M_3) [6]. The drugs (0.002-0. 2 mg/kg) were infused intravenously. The data were analyzed statistically by the method of direct differences [1].

RESULTS

After right-sided vagotomy the initial heart rate (HR) was 77.5±7.3 beats/min. The vagus nerve stimulation produced bradycardia associated with synchronization of the vagal and cardiac rhythms, so that every train of vagal pulses was accompanied by a single heart beat. The upper and lower boundaries of the synchronization range were determined (Table 1). The width of this range reflected the degree of VCE synchronizing component. The difference between the initial HR and the upper boundary of the synchronization range corresponded to the tonic component of VCE. The total value of VCE was measured by the sum of both components (Table 1).

The M_1 receptor antagonist pirenzepine in a dose of 0.002 mg/kg increased VCE caused by 3-, 6-, and

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9-pulse stimulation by 12.7, 10.3, and 7.4%, respectively. This effect was due to the enhancement of both VCE components (Fig. 1), but to a greater extent the synchronizing component. For instance, under conditions of 3-pulse stimulation the synchronizing component increased by 24.6%, while the inhibitory tonic component by only 10%. Higher doses of pirenzepine suppressed vagal regulation of myocardial activity probably due to atropine-like effects of cumulative doses. This explanation is in line with the finding that in a dose of 10^{-6} mol/kg pirenzepine extends the spectrum of its blocking action with respect to differently located muscarinic receptors [11].

The M_2 antagonist gallamine in a dose of 0.02 mg/kg reduced VCE caused by 3-, 6-, and 9-pulse stimulation by 14.7, 8.8, and 8.2%, respectively. This effect was due to a decrease in the tonic component by 17.2, 12.8, and 10.4% , respectively, while the range of synchronization of vagal and cardiac rhythms remained unchanged. When administered in a higher dose (0.2 mg/kg), gallamine more strongly inhibited the tonic component (Fig. 1) and suppressed the synchronizing (component of VCE by 52.3, 30.4, and 33.6% for 3-, 6-, and 9 pulse stimulation, respectively.

The M_3 antagonist 4-DAMP in a dose of 0.002 mg/kg increased the synchronizing component by 11.9, 15.0, and 14.4% at stimulation with 3, 6, and 9 pulses, respectively. A higher dose (0.02 mg/kg) of the drug increased this component by 21.5, 18.8, and 11.5%, respectively. The tonic inhibitory component was affected only by a dose of 0.2 mg/kg. The total amplitude of VCE after this dose decreased by 7.4, 8.9, and 9.5% at 3, 6, and 9 pulses, respectively (Fig. 1), and its tonic component decreased by 8.9, 9.2, and 10.7%, respectively.

These data suggest different role of various muscarinic receptor subtypes in the regulation of the VN chronotropic influence. A dose-dependent suppression of VCE was caused by M_2-receptor blockade, while M_1-receptor blockade increased its amplitude. These findings support the assumption that different cardiotropic effects can be mediated by different muscarinic receptor subtypes. It was reported that the inhibitory and stimulatory effects of acetylcholine on atrial contractility are realized through different types of muscarinic receptors [8].

Potentiation of the VN chronotropic effect after blockade of M_1 receptors can be attributed to several processes. It is known that threshold doses of atropine potentiate VCE. This was revealed in experiments on rats with VN stimulation [10] and confirmed by clinical data on the amplitude of vagal sinus arrhythmia [14]. This effect is due to blockade of presynaptic muscarinic cholinergic receptors on VN terminals suppressing the acetylcholine release upon vagal stimulation via the negative feedback mechanism. A similar mechanism can underlie the effects of pirenzepine. In nanomolar concentrations it increases the VN stimulation-induced release of acetylcholine by 50-100% [10]. On the other hand, the effect of pirenzepine can be explained by facilitated synaptic transmission in the parasympathetic ganglia. It was shown that low doses of acetylcholine enhance, while high doses suppress transmission in the intramural ganglia of frog myocardium [3]. Finally, the effect of pirenzepine can be attributed to changes in the intracellular calcium concentration. Activation of M_1 receptors leads to calcium accumulation in cardiomyocytes [15] which can be prevented by pirenzepine. The decrease in intracellular calcium concentration delays diastolic depolarization of pacemaker cells, thus contributing to potentiation of VCE.

The VCE components show different sensitivity to muscarinic receptor ligands. Thus, the nonselective M-receptor blocker methacine decreases the synchronizing but not tonic component [4]. On the other hand, the cholinergic agonist pilocarpine reduces the tonic inhibitory effect of the VN stimulation leaving the synchronizing component unaffected [2]. These data suggest that different VCE components are mediated by different subtypes of muscarinic receptors, which are distinguished by their affinity to the corresponding ligands. This suggestion is supported by our findings that the blockade of M_1- and M_3-receptors primarily

TABLE 1. Initial Dynamics of the Vagal Chronotropic Effect and its Components

Index, beats/min	Number of pulses (stimuli)		
	3	6	9
Synchronization range boundaries			
upper	120.3±5.6	104.5±6.1	98.2±5.8
lower	106.8±5.9	83.8±6.2	76.3±6.3
Width of synchronization range	13.5±1.5	20.7±1.7	21.9±1.7
Tonic component of VCE	57.2±5.6	69.4±5.8	79.3±6.1
Total amplitude of VCE	70.7±6.8	90.1±7.4	100.2±8.4

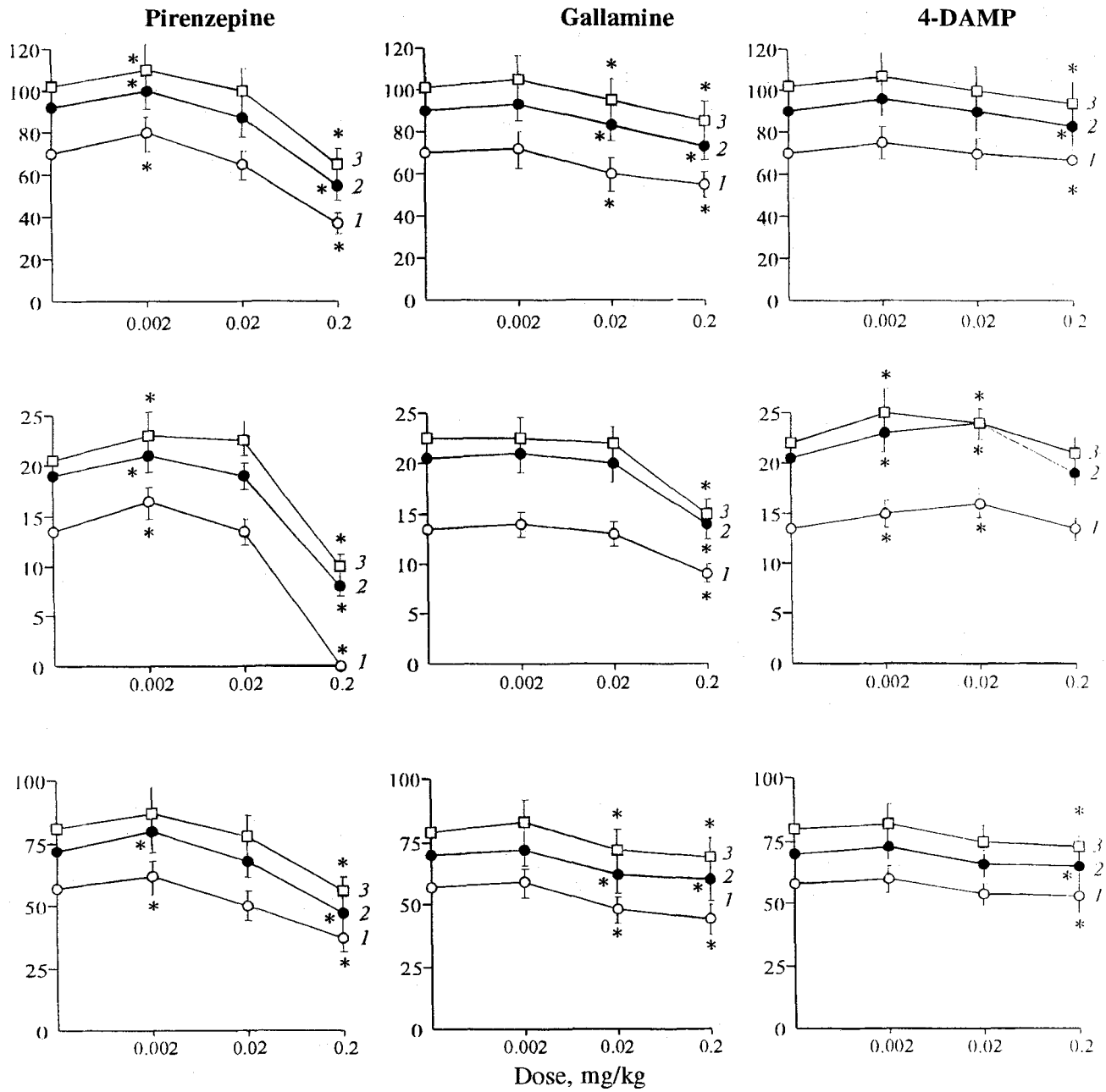

Fig. 1. Dynamics of the vagal chronotropic effect (*a*) and its synchronizing (*b*) and tonic (*c*) components during conditions blockade of different muscarinic cholinergic receptor subtypes. 1:3 pulses; 2:6 pulses; 3:9 pulses; *$p<0.05$ in comparison with the initial value. Ordinates: beats/min.

affects the synchronizing component of the VCE, while the tonic inhibitory component is more sensitive to M_2-receptor blockers and decreases at a gallamine concentration an order of magnitude lower than that necessary for the suppression of synchronization of the vagal and cardiac rhythms.

From our results it can be concluded that synchronization of the vagal and cardiac rhythms can be potentiated via activation of M_2-receptors and suppressed via M_1 and M_3-receptors coupled with different cell effector system. It is known that M_1- and M_3-receptors are coupled to stimulation of phosphoinositide hydrolysis, while the effects of M_2-receptor activation are realized through inhibition of adenylate cyclase [7].

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