METHODS

Mathematical Analysis of Subtypes of Muscarinic Receptors Modulating Heart and Respiratory Rhythms in Rats

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Mathematical analysis of the data obtained in experiments on the whole organism revealed that blockade of M_2-cholinergic receptors increased both heart and respiratory rates. Blockade of M_1-cholinergic receptors alleviated tachycardia induced by M_2-receptor blockade.

Key Words: *muscarinic receptors*; *heart rate*; *respiratory frequency*; *mathematical analysis*

The effect of numerous drugs is mediated by muscarinic receptors. There are several muscarinic receptor subtypes, which are abundantly spread over the organism. The modulating effects of various subtypes of muscarinic receptor are exceptionally variable and affect many physiological systems. This sometimes reduced selectivity of muscarinic receptor antagonists. This selectivity can be improved via the use of preparations with optimum selectivity profile for a particular pharmacological effect. This requires understanding of physiological role of different muscarinic receptor subtypes. However, numerous obstacles arise on this way. The main problem is the absence of ligands possessing high specificity for a particular muscarinic receptor subtype. This particularly concerns preparations penetrating the blood-brain barrier and affecting the central nervous system.

Here, a multiple linear regression analysis was applied for predicting the most likely receptor composition from experiments with ligands possessing medium selectivity to muscarinic receptor subtypes. Our aim was to evaluate the role of various muscarinic receptor subtypes in the regulation of cardiac and respiratory rhythms.

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MATERIALS AND METHODS

Experiments were carried out on albino rats weighing 180-240 g. Activity of muscarinic receptor antagonists were evaluated by their half-effective doses (ED_{50}) preventing pilocarpine- and arecoline-induced tremor, pilocarpine-induced salivation, or producing changes in heart rate (HR) and respiratory rate (RR). The rats were injected intramuscularly with arecoline (17 mg/kg) or pilocarpine (75 mg/kg) in the tremor test, and pilocarpine (35 mg/kg) in the salivation test. Muscarinic antagonists were injected subcutaneously 30 min before tests (4-5 increasing doses). The results were presented in the alternative form (yes-or-no effect). The presence of tremor and salivation in tests with pilocarpine and arecoline was assessed visually. The increases in HR and FR were considered significant, if these parameters changed by 15% or more. HR was evaluated by ECG recorded using a RM-6000 polygraph. Respiration was recorded using tetrapolar impedance plethysmograph coupled to a PVCh-01 polygraph. For these purposes, needle electrodes were placed on the back of rat thorax (current electrodes at the rostral and caudal edges of the thorax and voltage electrodes between them). ED_{50} was determined by probit analysis [5]. Commercial muscarinic receptor antagonists were used in the study.

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Mathematical processing of experimental data included a stepwise linear regression analysis and analysis of variance [2].

RESULTS

All test muscarinic antagonists increased both HR and RR (Table 1). For evaluation of the role of blockade of individual muscarinic receptor subtypes in the modulation of HR and RR, the efficacy (ED_{50}) of muscarinic receptor antagonists determined by their effects on HR and RR and in tests with muscarinic receptor antagonists were compared. It was previously established that pilocarpine- and arecoline-induced tremor and pilocarpine-induced salivation characterize the interaction of ligands with M_1-, M_2-, and M_3-receptors, respectively [3].

Experimental data were approximated by linear equations of the following type:

$$y=a_0+a_1x_1+a_2x_2+a_3x_3,$$

where y is ED_{50} of muscarinic receptor antagonist increasing HR and RR; x_1, x_2, and x_3 are ED_{50} for prevention of pilocarpine- and arecoline-induced tremor, and pilocarpine-induced salivation, respectively; a_0-a_3 are coefficients.

The stepwise linear regression analysis of the experimental data gave the following expressions, which characterize the role of blockade of different muscarinic receptor subtypes in HR and RR modulation, respectively:

$$y_1=4.1-6.2x_1+3.7x_2, \quad \textbf{(1)}$$
$$y_2=-0.2+1.1x_2. \quad \textbf{(2)}$$

Determination coefficients R^2, which show the accuracy of approximation with the linear regression model, were 0.95 and 0.97 for equations **(1)** and **(2)**, respectively.

According to equation **(1)**, y_1 increases with x_2, and decreases with x_1. Moreover, y_1 does not depend on x_3, which is absent in the equation. It can be concluded from the equations that the effects of muscarinic antagonists on HR are mediated via M_1- and M_2-receptor subtypes. Blockade of M_2-receptors produced an increase in HR, while blockade of M_1-receptors abolished this effect. Analysis of variance performed for equation **(1)** revealed that the relative weights of terms containing x_1 and x_2 were 20.5% and 79.5%, respectively.

According to equation **(2)**, y_2 is linearly proportional to x_2. Thus, the observed increase in RR was due to M_2-receptor blockade.

Equation **(1)** suggests that the increase in HR produced by muscarinic receptor antagonists is directly proportional to the degree of M_2-receptor blockade and inversely proportional to the degree of M_1-receptor blockade.

Cardiac muscarinic receptors, whose excitation results in bradycardia and weaker contractions, belong to M_2-receptor subtype [1]. Stimulation of M_2-receptors induces hyperpolarization in cardiomyocytes, which suppresses pacemaker activity. Thus, blockade of M_2-receptors by muscarinic receptor antagonists stimulates spontaneous activity of the sinoatrial and atrioventricular nodes and induces tachycardia. Apart from postsynaptic muscarinic receptors, presynaptic M_1-receptors are also found in chicken and guinea pig atria. Excitation of these receptors inhibits the acetylcholine release [6]. If preganglionic M_1-receptors are present in rat heart, the decrease in HR after blockade of M_1-receptors, which is predicted by equation **(1)**, can be easily explained by similar presynaptic mechanism.

According to equation **(2)**, blockade of M_2-receptors increases RR. It was shown that muscarinic receptors localized in rat lungs belong to the M_2- and M_3-subtypes [7]. Pulmonary M_2-receptors inhibit the release of acetylcholine from postganglionic cholinergic endings, while M_3-receptors are responsible for contraction of airway smooth muscles. Thus, the blockade of M_2-receptors stimulates acetylcholine release from nerve endings, which increases smooth muscle tone in the airways via stimulation of M_3-receptors. Airway constriction leads to hypercapnia, which in its turn increases RR via a reflex mechanism.

It should be noted that regulation of HR and RR involves all levels of the central control. Muscarinic receptors, as well as receptors specific to other neurotransmitters, are present in various brain regions.

TABLE 1. Pharmacological Activity (ED_{50}) of Various Muscarinic Receptor Antagonists in Rats ($M\pm m$)

Tests	Atropine	Amedine	Benactyzine	Glypine	Pentiphan	Cyclodol
HR (heart rate)	0.92±0.25	5.6±0.7	8.9±1.6	0.10±0.01	19.0±2.4	56.0±8.0
RR (respiratory frequency)	1.13±0.15	0.62±0.73	3.7±0.6	1.8±1.1	22.6±2.6	22.9±2.5
Arecoline-induced tremor	1.32±0.10	3.58±1.05	2.67±0.20	0.50±0.07	20.00±3.61	22.40±3.38
Pilocarpine-induced tremor	1.24±0.13	1.38±0.18	2.25±0.36	0.23±0.08	9.20±0.91	5.42±0.38
Pilocarpine-induced salivation	1.30±0.16	2.30±0.24	2.24±0.30	0.26±0.04	5.60±0.43	4.1±0.4

Some muscarinic antagonists poorly penetrating the blood-brain barrier because of their structure produce no dose-dependent increase in HR [4]. This probably attests to the presence of a central component in the development of tachycardia induced by M-antagonists. Similar central effect of cholinolytic drugs on RR can also be hypothesized.

Thus, a multiple linear regression model is a useful mathematical tool to analyze which muscarinic receptor subtype relates to a specific pharmacological response. However, additional methods are required for evaluation of M-receptor localization.

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