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PHYSIOLOGY

Effect of a Tetraalkylammonium Derivative of 6-Methyluracil on the Intensity of Evoked and Spontaneous Release of Acetylcholine from Motor Nerve Terminals

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Inhibitors of cholinesterases are important physiologically active substances. Some of them are drugs and are used to increase the efficacy of synaptic transmission of excitation in cholinergic synapses because they prolong the stay of the neurotransmitter molecules in the synaptic cleft and, hence, increase the probability of repeated activation of receptors of the endplate [1]. It is also known that synaptic transmission is facilitated upon inhibition of only part of acetylcholinestrase (ACE), whereas prolonged inactivation of a considerable part of the enzyme suppresses synaptic transmission (a curare-like effect) [2]. This effect may have pre- or postsynaptic mechanisms. The postsynaptic mechanism may include stable depolarization of the endplate due to the action of endogenous acetylcholine, which results in inactivation of sodium channels of the membrane of muscle fiber and/or desensitization of postsynaptic cholinergic receptors. The presynaptic mechanism of the curare-like effect may be based on the disruption of release of acetylcholine quanta due to activation of presynaptic cholinergic receptors that control the neurotransmitter exocytosis [3, 4]. Clinical use of all known ACE inhibitors is limited because of their very small "pharmacological safety" expressed as the ratio between the lethal and effective doses (LD_{50}/ED_{50}), which, for these drugs, does not exceed 5.0. Hence, from the toxicological viewpoint, they both "heal" and "kill" at practically the same doses. To extend the use of ACE inhibitors in medical practice, it is necessary to develop new substances with high anti-ACE activity and pharmacological safety [5]. Hence, a novel class of ACE inhibitors, tetraalkylammonium derivatives of 6-methyluracil, synthesized in the Arbuzov Institute of Organic and Physical Chemistry may be promising [6, 7]. A specific feature of the effect of these compounds in mammals in vivo, as compared to traditional anti-ACE drugs, such as organophosphate inhibitors, carbamates and onium salts, is a large range between the doses that cause paralysis of muscles of extremities during the functional load and the lethal doses that block respiratory muscles ($LD_{50}/ED_{50} > 50$) [8, 9]. The most effective tetraalkylammonium derivative of 6-methyluracil is 1,3-bis[5(diethyl-*o*-nitrobenzylammonium)pentyl]-6-methyluracildibromide (substance no. 547), whose coefficient of pharmacological safety is about 300 (it varies in different animal species) [8, 9]. Previously, in neuromuscular preparations of rat motor and respiratory muscles in vitro, we found differences in the efficacy of substance no. 547 on the amplitude–time parameters of miniature endplate potentials (MEPP) and currents [10, 11]. In this work, to evaluate the effect of substance no. 547 on the state of the presynaptic part of excitation transmission, we studied the intensities of evoked and spontaneous quantum release of the neurotransmitter in the synapses of muscles with different functions: motor muscles (the "rapid" m. extensor digitorum longus (m. EDL) and "slow" m. soleus) and muscles of the diaphragm ("mixed"; hereinafter, diaphragma) in the presence of substance no. 547.

The experiments were performed with isolated neuromuscular preparations of white outbred rats of both sexes weighing 250–300 g. An isolated muscle with an efferent nerve was placed in the experimental chamber and perfused with carbogen-aerated (95% O_2 and 5% CO_2) Ringer–Krebs solution, which contained (in mM): NaCl, 120.0; KCl, 5.0; $CaCl_2$, 2.0; $MgCl_2$ 1.0; $NaHCO_3$, 11.0; NaH_2PO_4, 1.0; and glucose, 11.0 (the pH of the solution was maintained at 7.2–7.4 at a temperature of 20.0 ± 0.5°C). We used substance no. 547 synthesized in the Laboratory of Chemical Biological

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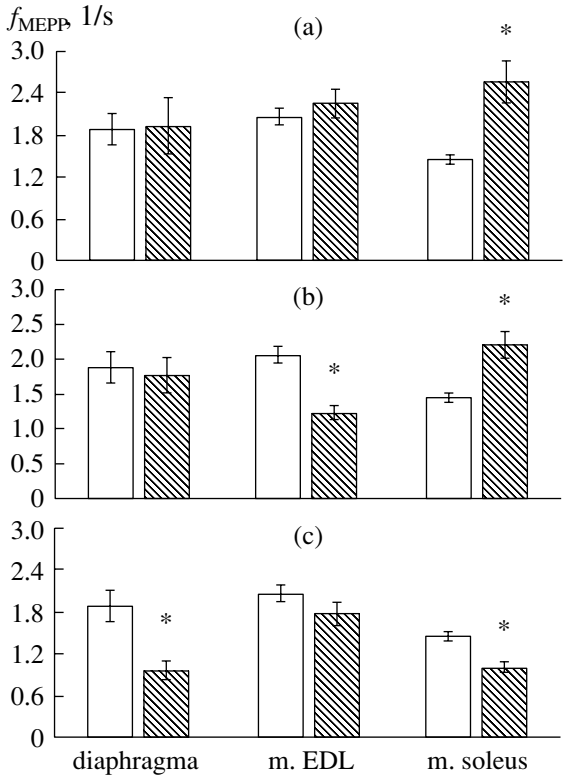

Fig. 1. Effect of substance no. 547 on the MEPP frequency in the synapses of muscles of different functional types: (a) 1×10^{-9} μ M; (b) 1×10^{-8} μM; (c) 1×10^{-7}μ M. White bars, control; hatched bars, in the presence of substance no. 547.

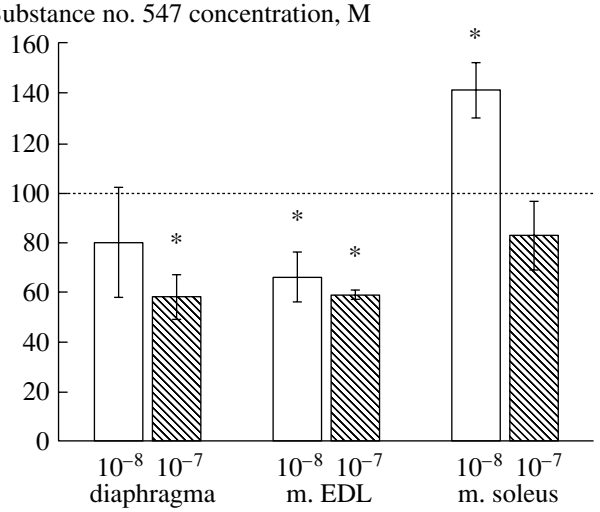

Fig. 2. Effect of substance no. 547 on the quantum content of EPPs in the synapses of muscles of different functional types. The ordinate axis shows the ratio between the quantum content of EPPs in the presence of the inhibitor and the quantum content in the control, which was taken to be 100%. White bars, 1×10^{-8} μM substance no. 547; hatched bars, 1×10^{-7} μM substance no. 547.

Studies of the Arbuzov Institute of Organic and Physical Chemistry, Kazan Research Center, Russian Academy of Sciences. The endplate potentials (EPP) and MEPPs induced by stimulation of the motor nerve with a frequency of 0.5 pulse/s were recorded with the use of the standard microelectrode technique. After preliminary amplification, the signals were recorded to a computer hard disk and analyzed with the use of an original software. The spontaneous quantum release of acetylcholine was estimated by the mean frequency and histograms of the distribution of interimpulse intervals of MEPPs. The quantum content of EPPs was evaluated under the conditions of a decreased Ca^{2+} concentration (0.6 mM) and increased Mg^{2+} concentration (6.0 mM) or by division of the mean EPP amplitude by the mean MEPP amplitude or by the "failure" method [12, 13].

Under the initial conditions, the intensity of spontaneous quantum secretion of the neurotransmitter in the m. soleus was significantly lower than in the diaphragma and the m. EDL. The mean frequency of MEPPs in the diaphragma was 1.9 ± 0.2 pulse/s ($n = 8$); in the m. EDL, it was 2.0 ± 0.1 pulse/s ($n = 7$); and in the m. soleus, it was 1.4 ± 0.1 pulse/s ($n = 6$, $p < 0.05$) (Fig. 1).

In the diaphragma, substance no. 547 at concentrations of 1×10^{-9} M and 1×10^{-8} M induced no significant changes in the MEPP frequency, which was 1.9 ± 0.4 pulse/s ($n = 8$, $p > 0.05$) and 1.8 ± 0.3 pulse/s ($n = 7$, $p > 0.05$), respectively; only an increase in the inhibitor concentration to 1×10^{-7} M decreased the spontaneous release of the neurotransmitter to 1.0 ± 0.4 pulse/s ($n = 6$, $p < 0.05$) (Fig. 1).

In the m. soleus, we observed an increase in the MEPP frequency in the presence of 1×10^{-9} and 1×10^{-8} M substance no. 547, whereas at higher concentrations of this substance, the MEPP frequency decreased to 1.0 ± 0.1 pulse/s ($n = 6$, $p < 0.05$). In the m. EDL, substance no. 547 had an effect only at concentrations no lower than 1×10^{-8} M, at which the mean frequency of MEPPs decreased to 1.2 ± 0.11 pulse/s ($n = 6$, $p < 0.05$) (Fig. 1). The data obtained indicate that the effective concentration of substance no. 547, which changes the intensity of spontaneous release of neurotransmitter quanta, was approximately two orders of magnitude higher in the respiratory muscle than in the locomotor muscles.

The mean quantum content of EPPs in the m. soleus, m. EDL, and diaphragma was 1.5 ± 0.2 ($n = 6$), 1.0 ± 0.1 ($n = 6$), and 2.0 ± 0.4 ($n = 6$), respectively. The variation of the initial quantum content in different synapses of the same muscle was very high; hence, under control conditions, no differences were found in the quantum content in different muscles. As with spontaneous secretion of quanta, the intensity of evoked release was less sensitive to substance no. 547 in the diaphragma than in locomotor muscles. In the diaphragma, a significant decrease in the quantum content (by 42%) was observed only in response to treatment with 1×10^{-7} M substance no. 547, whereas in the m.

EDL, the quantum content decreased by 34% upon application of the substance at a concentration of 1×10^{-8} M. In the m. soleus, the inhibitor also had an effect at a concentration of 1×10^{-8} M; however, this effect was opposite (Fig. 2).

Thus, the concentrations of substance no. 547 that changed the intensity of spontaneous quantum release and quantum content of EPPs were considerably higher in synapses of the respiratory muscles than in those of locomotor muscles.

The results of our experiments provide no information on the exact molecular mechanisms responsible for different sensitivities of motor nerve terminals of different muscles to substance no. 547; however, we believe that the considerably smaller sensitivity of evoked neurotransmitter release in the respiratory muscle to the inhibitor contributes to an increased index of pharmacological safety of this substance **in in** vivo experiments. The fact that spontaneous release is also more resistant to the action of the drug in the synapses of the diaphragm suggests that the mechanisms of the lower sensitivity of nerve terminals of the respiratory muscle should be studied at the stages of neurotransmission common for spontaneous and evoked release.

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SPELL: 1. diaphragma

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