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PHYSIOLOGY

Specific Inhibitory Effects of the Alkylammonium Derivative 6-Methyluracil on Acetylcholinesterase of Smooth and Striated Muscles in Rats

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Today, the inhibitors of acetylcholinesterase (AChE) are widely used in medicine for pharmacological correction of synaptic failure underlying Alzheimer's disease, myasthenia gravis, and other forms of pathological muscular weakness, and for stimulation of smooth muscles of the intestine and bladder in order to increase the tone and stimulate peristalsis [1, 2]. Moreover, partial inhibition of AChE activity is used in anesthesiology for acceleration of unblocking neuromuscular excitation transmission in patients induced by administration of muscular relaxants of the antidepolarizing type, such as curare and its analogues [3]. In these cases, the efficacy of anti-AChE drugs is based on their ability to potentiate the effects of endogenous acetylcholine (ACh) due to a decrease in the rate of enzymatic hydrolysis of ACh. Under these conditions, extension of the duration of the presence of transmitter molecules in the synaptic cleft leads to excitation of other cholinoreceptors and increases the amplitude of the excitating postsynaptic potential (EPSP). In diseases related to decreases in the density of functionally active cholinoreceptors on the postsynaptic membrane or intensity of transmitter secretion such as myasthenia gravis, Lambert–Eaton syndrome, Alzheimer's disease, etc. [4, 5], an increase in the EPSP amplitude after AChE inhibition recovers the capability of EPSP of evoking action potential in postsynaptic cell. In atony of the intestine and bladder, an increase in the EPSP amplitude results in strengthening contraction of smooth muscular elements, an elevation of the tone of organs, and enforcement of peristalsis.

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However, extensive clinical application of the AChE inhibitors should be very careful, because, in addition to the effect on the organ the function of which requires pharmacological correction, they simultaneously inactivate the enzyme in other organs, which do not require any correction. This results in multiple side effects. For example, the use of anti-AChE drugs for treatment of syndromes of pathological muscular weakness may be associated with impairments of normal functioning of the heart and smooth muscles of the vessels and intestine [6].

The AChE inhibitors that would be effective in synapses of specific tissues should have no above-mentioned problems. However, these inhibitors have not been described until now.

We have previously described [7] a new class of the AChE inhibitors, alkylammonium derivatives of 6-methyluracil. One of them, namely 1,3-bis[5(diethyl-o-nitrobenzylammonium)pentyl]-6-methyluracildibromide (C-547), had a selective effect on AChE of the skeletal muscles compared to the respiratory muscles. We have also found that compound no. C-547 inhibits AChE in the myocardium at concentrations, which are two orders of magnitude higher than those that completely inhibit AChE in the skeletal muscles [8]. C-547 contains in its structure quaternary nitrogen that troubles its transition across the blood–brain barrier; therefore, the most probable area of its application is treatment of syndromes of pathological muscular weakness and smooth muscle atonia. In this study, we compared the efficacies of C-547 and one of AChE inhibitors widely used in medicine, kalymin, in synapses of the striated muscles and smooth muscles.

The experiments were performed in isolated preparations of the bladder and musculus extensor digitorum longus (m. EDL) of white underbred rats of both sexes (body weight, 250–300 g). The rats were decapitated under ester anesthesia, and the organs were dissected. The isolated bladder and muscle were put into Ringer–Krebs solution consisting of (in mmol/L): NaCl, 120.0; KCl, 5.0; $CaCl_2$, 2.0; $MgCl_2$, 1.0;

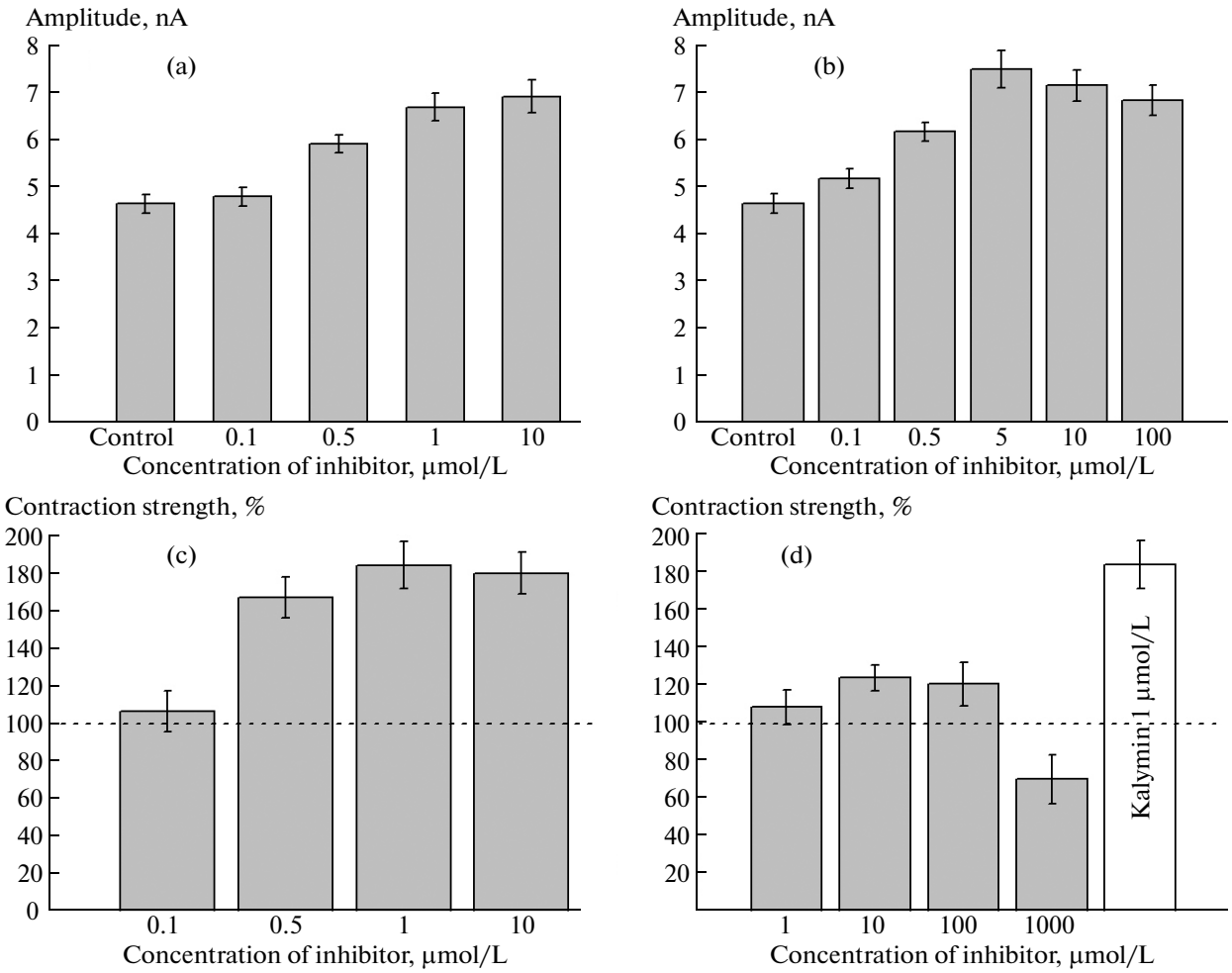

Fig. 1. Effects of the AChE inhibitors on the MEPC amplitude in the m. EDL and on the contraction strength of the bladder strips of rats. (a) m. EDL, kalymin; (b) m. EDL, C-547; (c) bladder, kalymin; (d) bladder, C-547. For MEPC, $n = 30$; for bladder preparations $n = 5$.

$NaHCO_3$, 11.0; NaH_2PO_4, 1.0; glucose, 11.0; pH 7.2–7.4 at the temperature of 20 ± 2°C. The bladder was cut into strips 2–3 mm in width and 10 mm in length and placed into a 20-mL dish filled with Ringer–Krebs solution and aerated with carbogen. The contraction response was measured using a medium-sensitive device (Ugo Basile, model no. 7004). Contractions were analyzed using the Virtual Chart Recorder 1024 × 768 computer software. Contractions of the bladder preparations were induced by addition of 100 μmol/L ACh into the incubation medium. Then, the preparations were washed with the solution without the neurotransmitter and allowed to recover the smooth musculature tone to the initial level. The following protocol was used for the next experiment: the preparations were incubated for 20 min in solutions containing various concentrations of the inhibitor and, after that, their responses to application of 100 μmol/L ACh were measured.

Miniature endplate currents (MEPCs) were measured using the standard microelectrode technique. In order to prevent spontaneous muscle contractions induced by AChE inhibition, the perfusion solution was supplemented with the Na^+ channel blocker tetrodotoxin at a concentration of 1 μmol/L.

The differences were considered significant at $p < 0.05$ according to Student's *t*-test. C-547 was synthesized in the Laboratory of Chemical and Biological Research, Arbuzov Institute of Organic and Physical Chemistry, Kazan Scientific Center of the Russian Academy of Sciences. Pyridostigmine bromide (kalymin) was obtained from Sigma–Aldrich.

We found that, in the control, the mean MEPC amplitude in synapses of the m. EDL was 4.6 ± 0.2 nA ($n = 30$). Contractions of smooth muscles in preparations in response to ACh application were 2.9 ± 0.2 g ($n = 5$). Similar to the AChE inhibitors, kalymin treatment resulted in a dose–dependent increase in the MEPC amplitude in the m. EDL at a concentration of 0.1 μmol/L ($n = 30$, $p < 0.05$) (Fig. 1a). The maximum elevation of the MEPC amplitude was observed at a

kalymin concentration of 1 μmol/L that was 144% of the control level ($n = 30$; $p < 0.05$). The threshold kalymin concentration inducing an enhancement of contraction strength in the bladder preparations was 0.1 μmol/L ($n = 5$; $p < 0.05$), which was similar to that observed in the striated muscle. The maximum increase by 85% was also observed at a concentration of 1 μmol/L ($n = 5$; $p < 0.05$) (Fig. 1c).

C-547 was much more effective than kalymin. A significant increase in the MEPC amplitude by 12% was observed at a concentration of 0.1 nmol/L ($n = 30$; $p < 0.05$). The maximum elevation of the current amplitude was observed at C-547 concentration of 5 nmol/L, which was 161% of the control level ($n = 30$; $p < 0.05$) (Fig. 1b). In the experiments performed with the bladder muscle, C-547 was ineffective even at a concentration of 1 nmol/L (109.0 ± 9.5%; $n = 5$; $p < 0.05$). Application of C-547 at a concentration of 10 nmol/L resulted in an increase in the strength of contraction of the bladder strip (124.6 ± 6.8%; $n = 5$; $p < 0.05$) (Fig. 1d). Elevation of the C-547 concentration up to 1 μmol/L decreased the contraction strength down to 70.7 ± 13.1% ($n = 5$; $p < 0.05$), i.e., to values lower than the control value.

Thus, the most effective concentrations of C-547 in the skeletal muscles were different from those observed in the smooth muscles by a factor of 2; however, these differences were not revealed for kalymin.

Our data show that C-547 is more effective in AChE inhibition in synapses of the striated muscles compared to synapses of the smooth muscles. This specific feature of C-547 indicates that the compounds of this class are promising for future studies as potential drugs for treatment of myasthenia gravis and inherited myasthenia-like states.

Future studies on the molecular mechanisms underlying the differences in the efficacy of AChE inhibition in synapses of various organs will allow the synthesis of compounds with even more pronounced organ- and tissue-specific properties.

What are the causes of the differences in the efficacy of C-547 in various organs? AChE is known to have several molecular forms, the ratio between which varies in different organs. Several processes underlie the variety of molecular forms of AChE. For example, several forms of alternative splicing of the AChE gene have been described [9]. Moreover, the differences have been reported in posttranslational modifications of AChE, including glycosylation and addition of some variants of "anchor" proteins [10]. We mentioned above that C-547 is the first candidate for the role of an organ-specific AChE inhibitor. However, the reasons for these selective effects of C-547 in different organs are presently unknown. In order to find the reasons for the specific sensitivity of AChE to C-547, we plan to focus at the analysis of differences in the ratio of particular molecular forms of AChE in the smooth and striated muscles.

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