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PHYSIOLOGY

Selective Blockade of Locomotor Muscles by Uracil-Containing Tetraalkylammonium Acetylcholinesterase Inhibitors

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The ratio between muscle-relaxing and lethal doses of onium cholinesterase inhibitors characterizes the selectivity of their effect on the locomotor muscles and the corresponding level of "pharmacological safety" (LD_{50}/ED_{50}). The greatest selectivity is characteristic of oxazyl (ambenonium), whereas the lowest selectivity is exhibited by the majority of cholinesterase inhibitors [1]. Taking into account the fact that the specific effect of cholinesterase inhibitors lasts insufficiently long, the search for ways of increasing the selectivity of their effect is a topical problem.

Previous studies [2, 3] showed that, among the tetraalkylammonium derivatives of 6-methyluracyl targeted at the "peripheral anionic site" of acetylcholinesterase (EC 3.1.1.7), there are compounds with a number of biochemical abnormalities, such as (1) selective, progressing in time inhibitory activity with respect to acetylcholinesterase ($k^0 = 7.6 \times 10^8$–3.5×10^9 M^{-1} min^{-1}) and (2) irreversible inhibition of acetylcholinesterase preparations of homeotherms and reversible inhibition of acetylcholinesterase preparations of poikilotherms. The presence or absence of abnormalities is determined by the structure of ammonium and *N*-heterocyclic fragments of molecules, as well as by the nitrogen atom of the uracil cycle with which the benzyldiethylamylammonium fragment is bound [3].

The above-mentioned characteristics of interaction between the tetraalkylammonium derivatives of 6-methyluracil and cholinesterases of various groups of animals *in vitro* [3] rise the question to which extent these characteristics are realized *in vivo* at the whole-body level (e.g., mouse and *Daphnia*).

Compounds 1–4 (table) in the form of aqueous solutions were injected intraperitoneally to unbred albino mice of both sexes weighing 19.0 ± 2.0 g or applied to the standard laboratory *Daphnia magna* culture at the age of 18 ± 6 h [4], which allowed the level of "ecological safety" of these compounds to be determined. The half-lethal doses (LD_{50}) in the case of mice (72 h of observations) and concentrations (LC_{50}) in the case of *Daphnia* (48 h of observations) were used as toxicity indices.

Half-effective doses (ED_{50}) in the functional test on a treadmill (Nihon Kohden, 1 km/h) [5], as well as the rate of development and the maximal duration of the muscle-relaxing effect, were used as indices of muscle-relaxing activity. The development of muscle-relaxing effect under the conditions of functional load is one of the key and well reproducible symptoms of the effect of anticholinesterase agents [1]. The compounds studied were injected to animals 5 min prior to the beginning of physical activity. The inability of mice (preliminarily adapted for running on a treadmill) to run on a treadmill for 30 min served as a criterion for muscle-relaxing activity of the compounds of interest. Similarly, LD_{50}, LC_{50}, and ED_{50} values were determined for the reference anticholinesterase preparations (proserin, oxazyl, and BW284c51).

Results were processed by means of variational statistics using Student's *t*-test, as well as with the use of the ToxCalc™ v.5.0.23E software (United States). The results are summarized in Tables 1 and 2.

As seen from the table, in experiments performed with mice, compounds 1–4 can be assigned to the category "highly toxic compounds" [6]. In experiments with *Daphnia*, the toxicity of these compounds was lower by seven orders of magnitude than the toxicity of their phosphorylated analogues [7], which allowed them to be classified with the "slightly toxic" compounds ($LC_{50} > 10.0$ μM) [8].

Although compounds 1–4 at sublethal doses caused the symptom of head bowing in rabbits, which is characteristics of curare-like compounds, hyperexcitement with subsequent muscle relaxation of extremities predominated in the net picture of poisoning.

We found that compounds 2–4, unlike compound 1, proserine, and BW284c51, exhibited a sufficiently high selectivity of the blocking effect on the locomotor muscles, unusual for cholinesterase inhibitors, which was

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Parameters of the biological activity of tetraalkylammonium derivatives of 6-methyluracil

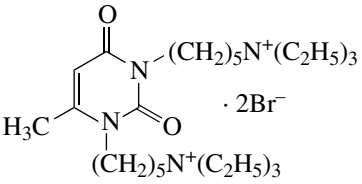
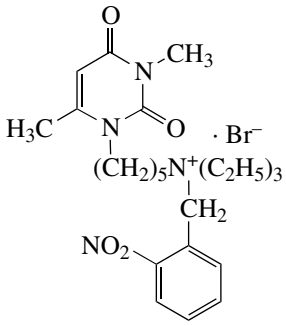
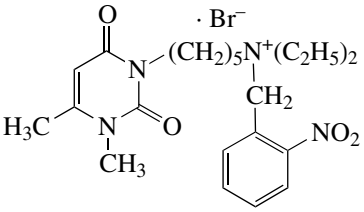
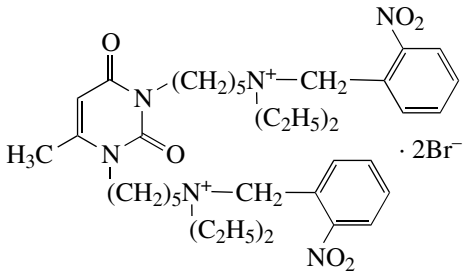
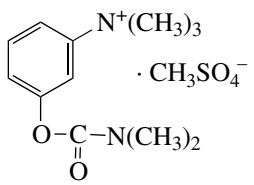
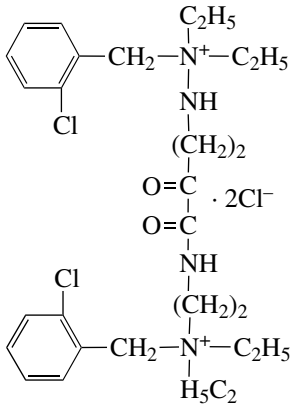
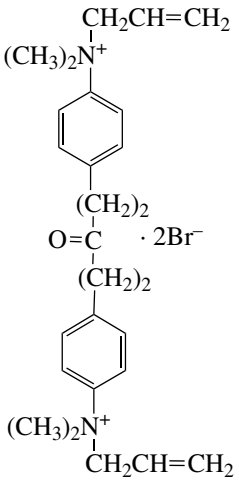
Compound	Toxicity		Effectiveness, ED_{50} mice, intraperitoneal injection, treadmill test, μM/kg (n = 32)	LD_{50}/ED_{50}
	LC_{50}, *D. magna*, μM (n = 90)	LD_{50}, mice, intraperitoneal injection, μM/kg (n = 24)		
(1) 	935.00*#&	1.69#	0.37#&	4.57#&
	779.17–1122.00	1.42–2.01	0.31–0.44	3.35–5.79
			Effect duration, <1 h	
(2) 	58.91*#	6.03*&	0.30#	20.10*#&
	50.35–68.92	5.34–6.82	0.27–0.34	16.37–23.83
			Effect duration, 1–2 days	
(3) 	64.29*	2.61*#	0.12*&	21.75*#&
	54.03–76.51	2.31–2.95	0.11–0.13	18.39–25.11
			Effect duration, <1 h	
(4) 	18.71*#&	1.17#&	0.03*&	39.00*#&
	15.33–22.82	1.04–1.31	0.03–0.04	34.33–43.67
			Effect duration, 4–5 days	
 Proserin	2.70#&	1.53#	0.39#	3.92#
	2.21–3.29	1.34–1.74	0.34–0.45	3.11–4.73
			Effect duration, 1 h	

Table (Contd.)

Compound	Toxicity		Effectiveness, ED_{50} mice, intraperitoneal injection, treadmill test, μM/kg (n = 32)	LD_{50}/ED_{50}
	LC_{50}, *D. magna*, μM (n = 90)	LD_{50}, mice, intraperitoneal injection, μM/kg (n = 24)		
 Oxazyl	119.30* 97.79–145.54	8.77*& 7.69–10.00	0.09*& 0.08–0.10 Effect duration, 1 h	97.44*& 71.88–123.08
 BW284c51	100.56* 82.43–122.69	2.12# 1.86–2.42	0.21# 0.18–0.24 Effect duration, 2 h	10.10# 8.07–12.13

Note: Differences from *proserin, #oxazyl, and & BW284c51 are significant ($p < 0.05$).

reflected on the parameter LD_{50}/ED_{50} (20.0–40.0; see table). The muscle-relaxing effect of compounds 1–4 developed within 5 min after injection; however, its duration significantly varied. For example, the effect of compound 2 (a nonselective acetylcholinesterase inhibitor [3]) was long-term (one to two days), whereas compound 3 (an isomer of compound 2, a selective acetylcholinesterase inhibitor [3]) had a short-term effect that lasted less than 1 h. The duration of the effect of compound 4 (the bisquaternary analogue of compounds 2 and 3, a selective acetylcholinesterase inhibitor [3]; laboratory code no. 547) reached five days (table). However, the effect of compound 1 (the tetraalkylammonium analogue, a nonselective acetylcholinesterase inhibitor [3]) was short-term (less than 1 h).

The expression and duration of selective disturbance of the locomotor function of animals by compounds 2 and 4 depended on the intensity of movements (the so-called use-dependent effect). For example, even a slight physical activity on a treadmill provoked a strong tremor in animals, which was then changed with convulsive muscular twitching of extremities, thereby excluding their coordinated locomotor activity for up to five days. However, the animals retained all the major reflexes and remained alive.

It is noteworthy that relatively high LD_{50}/ED_{50} values (>10.0) are characteristic of compounds 2–4, whose activity spectrum combined a pronounced anticholinesterase activity (manifested at low doses) and the curare-like symptoms (manifested at sublethal

doses). Similar symptoms and a high LD_{50}/ED_{50} ratio is characteristic of oxazyl, a well-known anticholinesterase agent with cholinolytic activity; however, it had a short-term muscle-relaxing effect (table).

Notably, the group of "pharmacologically safe" compounds ($LD_{50}/ED_{50} > 10.0$) includes both selective (compounds 3 and 4 [3]) and nonselective (compound 2 [3] and oxazyl [1]) acetylcholinesterase inhibitors. On the other hand, both the selective (BW284c51) and nonselective (compound 1 [3] and proserin [1]) acetylcholinesterase inhibitors have a low LD_{50}/ED_{50} ratio (<10.0; table). These facts show that the problem of pharmacological safety of cholinesterase inhibitors does not boil down solely to differences in the pharmacological properties of compounds. Possibly, the differences in the "safety factors" of neuromuscular transmission in muscles of different functional types (diaphragm/locomotor) provide favorable conditions for realizing opposite pharmacodynamic effects of compounds 2–4 (anticholinesterase/cholinolytic), which enables the functioning of certain muscles (respiratory) and causes blockage of others (locomotor), with subsequent selective and long-term blockade of the locomotor function.

The data obtained in this study are indicative of a key role of the 6-methyluracil fragment and nature of cyclic substituents at the tetraalkylammonium pharmacophores in providing for additional increase in the selectivity of effect of ligands both on acetylcholinesterase preparations *in vitro* [2, 3] and in entire animals (*in vivo*). This allows these compounds to be regarded as a promising class of cholinesterase inhibitors combining the properties of increased "pharmacological" ($LD_{50}/ED_{50} > 10.0$) and "ecological" ($LC_{50} > 10.0$) safety.

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