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ALKALOIDS OF *Anabasis aphylla* AND THEIR CHOLINERGIC ACTIVITIES*

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A comparative study has been made of the anticholinesterase activities of the methiodides and hydrochlorides of anabasine, lupinine, anabasamine, and methyl aphyllinate, which have proved to be reversible inhibitors of two types of cholinesterases.

Among the derivatives of various alkaloids [1-3], compounds have been found that undergo catalytic hydrolysis under the action of cholinesterases (ChEs) and may therefore be assigned to the synthetic substrates of these enzymes. However, other derivatives of this series of substances cause inhibition of ChE activity, i.e., are ChE inhibitors.

By various methods [4-10], 10 alkaloids have been isolated from the plant *Anabasis aphylla* growing in the Central Asian region. Structural differences and the presence of reactive groupings, and also conformational features of these alkaloids, may play an important role in the manifestation of a biological effect.

We have studied the dependence of the cholinergic activity of anabasine, lupinine, anabasamine, and methyl anaphyllinate on their structural features.

Starting from the structures of these alkaloids, it is not difficult to assume that as the result of their interaction with a ChE an enzyme-sorption complex is formed with the anionic point of the ChE located on the active surface of such a hydrolase. The screening of this section of the alkaloid molecule interferes with the access of the substrate to the active center, a consequence of which is a decrease in the catalytic hydrolysis of acetylcholine under the action of the ChE. All the alkaloids under investigation are nitrogen-containing heterocycles and their structure is close to the ammonium "head" of the ChE substrate acetylcholine. At physiological pH values, these groupings are capable of undergoing protonation and, consequently, of ion–ion interaction with the anionic point of the ChE. The inhibition of the activities of acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) by the alkaloids did not depend on the time of incubation, which shows the reversible nature of the inhibition of the activity of the hydrolases under study.

Table 1 gives the constants of reversible inhibition of AChE and BuChE by the alkaloids of *Anabasis aphylla*. It can be seen that, with the exception of the alkaloid anabasamine, these substances are inhibitors of moderate strength. Among the alkaloids studied, lupinine hydrochloride is a strong inhibitor of BuChE, and anabasine methiodide of AChE. While the former inhibits the activity of BuChE by the mixed type of inhibition, the latter interacts with AChE without competing for the substrate-binding section.

Anabasine hydrochloride, methiodide, and sulfate inhibit AChE activity equally strongly. In the case of BuChE, anabasine hydrochloride exhibits a greater effectiveness than its methiodide and sulfate. Lupinine – quantitatively the second alkaloid of *Anabasis aphylla* – inhibits the activities of both types of ChE predominantly by the mixed type of action. The hydrochloride is most active in relation to BuChE, while on interaction with AChE its methyl iodide analog effectively fulfills this role. The ratio of inhibition constants for the action of the methiodide and the hydrochloride on AChE and BuChE is 7.1; i.e., lupinine hydrochloride inhibits the catalytic activity of AChE more effectively than the methiodide on interaction with BuChE [sic].

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TABLE 1. Anticholinesterase Activities of the Alkaloids of *Anabasis aphylla*

Alkaloid	AChe			BuChE		
	K_i	pK_i	t/i *	K_i	pK_i	t/i
Anabasine						
hydrochloride	$3.24 \cdot 10^{-4}$	3.49	n	$1.98 \cdot 10^{-4}$	3.70	c
methioide	$3.10 \cdot 10^{-4}$	3.50	m	$1.48 \cdot 10^{-3}$	2.83	c
sulfate	$3.3 \cdot 10^{-4}$	3.48	n	$1.8 \cdot 10^{-3}$	2.74	n
Lupinine						
hydrochloride	$4.8 \cdot 10^{-4}$	3.31	m	$1.80 \cdot 10^{-4}$	3.72	m
methioide	$1.73 \cdot 10^{-4}$	3.76	n	$3.42 \cdot 10^{-3}$	2.48	m
Epilupinine						
hydrochloride	$1.04 \cdot 10^{-3}$	2.9	c	$1.9 \cdot 10^{-4}$	3.72	c
methioide	$2.04 \cdot 10^{-3}$	2.69	c	$2.46 \cdot 10^{-4}$	3.06	c
Anabasamine						
hydrochloride	$3.18 \cdot 10^{-4}$	3.49	n	$8.2 \cdot 10^{-3}$	2.08	n
methioide	$5.1 \cdot 10^{-5}$	4.29	n	$4.4 \cdot 10^{-4}$	3.35	n
Methyl anaphyllinate						
hydrochloride	$4.6 \cdot 10^{-4}$	3.33	m	$1.8 \cdot 10^{-3}$	2.74	m
methioide	$7.9 \cdot 10^{-4}$	3.10	m	$1.2 \cdot 10^{-3}$	2.92	m

*t/i) Type of inhibition: m) mixed, c) competitive, n) noncompetitive.

The epimer of lupinine, epilupinine (in the form of its hydrochloride and methiodide), exhibits a greater inhibiting activity in its action on BuChE than on AChE. Their inhibition constants differ by more than an order of magnitude, and the inhibition of the enzymes has an exclusively competitive nature.

Anabasamine, an alkaloid with three condensed heterocyclic rings, affects the catalytic activity of AChE more strongly than that of BuChE. Anabasamine methiodide is a stronger inhibitor of AChE and BuChE than the hydrochloride. It must be mentioned that anabasamine methiodide exhibits the most pronounced inhibiting activity among the alkaloids of *Anabasis aphylla* and is 8.6 times more effective in interaction with AChE than with BuChE. This activity is even higher in the interaction of anabasamine hydrochloride with both AChE and BuChE.

In the investigation of the influence of the hydrochloride and methiodide of methyl anaphyllinate, it was established that AChE is more sensitive to these compounds than the serum enzyme BuChE. The inhibition of the activity of BuChE by these substances has an almost uniform nature and is analogous to the action of anabasine sulfate on these enzymes.

There is no doubt that an important factor for the sorption of alkaloids is the spatial correspondence of their structures to the active centers of AChE and BuChE. In addition to Coulomb forces, an important role for binding with them is played by the hydrophobic interaction of the cyclic structures of the alkaloids with the hydrophobic environment of the anionic point of a ChE. The inhibition constants obtained permit the conclusion that the strength of the anticholinesterase action depends not only on the structure of the alkaloids and their conformational features but also on the type of ChE.

A comparison of the structures and anticholinesterase actions of the alkaloids of *Anabasis aphylla* shows that AChE has a broader area in the region of the anionic point, which is capable of accomodating the alkaloid anabasamine, consisting of three interlinked heterocycles. As compared with AChE, BuChE possesses a smaller capacity for sorbing the anabasamine rings. These facts suggest that when anabasamine is sorbed on the active surface of a ChE the anticholinesterase activity rises not only through Coulomb interaction but also as a consequence of an increase in hydrophobic binding with the analogous formations of the enzyme located close to the anionic point.

Thus, a study has been made of a group of reversible ChE inhibitors – alkaloids from *Anabasis aphylla* – that can be used as new tools for the comparative investigation of features of the topography of the active center of ChEs from various sources.

EXPERIMENTAL

As the sources of enzymes we used purified preparations of human blood erythrocyte AChE (E.C. 3.1.1.7) and of horse blood serum BuChE (E.C. 3.1.1.8) with specific activities of 1.2 and 9.6 units produced by the Perm Scientific Research Institute of Vaccines and Sera.

The catalytic activities of the ChEs were determined by Ellman's colorimetric method [11] using acetylcholine iodide as the substrate.

The effectiveness of the alkaloids as reversible inhibitors was evaluated from the magnitudes of the inhibition constants K_i [12].

In Table 1, for convenience of comparing the kinetic parameters, the effectiveness of inhibition is represented in the form of the negative logarithm of the inhibition constant, pK_i.

The methiodides and hydrochlorides of the alkaloids were obtained by the action of methyl iodide or of hydrogen chloride on their solutions in methanol.

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