A solution of 4.1 g of the heptapeptide in 11 ml of chloroform was deposited on a column (3 × 35 cm) containing Kieselgel 60 (Merck, 230-400 mesh) and was eluted with the chloroform–methanol (9.5:0.5) system. Fractions containing the chromatographically homogeneous substance (monitoring by TLC) were collected. The solvents were driven off in vacuum. The yield of product was 3.1 g (76% of theory), R_f 0.31-0.35 (system 2), $[\alpha]_D^{20}$ −72.0° (s 1; MeOH).

10. Preparation of H-MetGluHisPheProGlyProOH. A solution of 3.0 g (2.9 mmole) of the protected heptapeptide of para. 9 in 24 ml of dimethyl sulfide and 12 ml of anisole was treated with 36 ml of trifluoroacetic acid and the mixture was kept at 30°C for 3 h. Then 100 ml of diethyl ether was added and the resulting precipitate was filtered off, washed with 50 ml of ether, and dried in vacuum. The product was dissolved in 30 ml of water and the solution was passed through a column (1 × 50 cm) with EDE-10p resin in the AcO^- form. The column was washed with 20 ml of water and the combined eluates were lyophilized. The yield of product was 2.1 g (88% of theory), $[\alpha]_D^{20}$ −68° (s 0.5; AcOH).

SUMMARY

A procedure has been developed for obtaining a modified ACTH 4-10 heptapeptide which is convenient for the preparation of the substance in relatively large amounts.

LITERATURE CITED

1. H. Van Riezen, H. Rigter, and D. De Wied, Behav. Biol., 20, 311 (1977).
2. M. A. Ponomareva-Stepnaya, V. N. Nezavibat'ko, L. V. Angonova, and I. P. Ashmarin, et al., Khim-Farm. Zh., 7, 790 (1984).
3. E. Schroeder and K. Lübke, The Peptides, Academic Press, New York (1965).

INTERACTION OF THE CHOLINESTERASES OF WARM-BLOODED ANIMALS WITH ESTERS OF N-(β-HYDROXYPROPYL)PIPERIDINES AND THE METHIODIDES

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The influence of esters of N-(β-hydroxypropyl)pyridines (alkyl radicals from CH_3 to C_4H_9) on the enzymatic activity of acetylcholinesterase (ACE) from human blood erythrocytes and on that of butyrylcholinesterase (BCE) from horse blood serum has been studied. At pH 7.5 and 25°C the majority of the piperidine derivatives inhibit reversibly (by the competitive type of inhibition) both ACE and BCE, their inhibiting properties depending only slightly on the presence of the corresponding alkyl radicals in the acid part of the molecule.*

The main types of cholinesterases — acetylcholinesterase (ACE) and butyrylcholinesterase (BCE) — differ from one another not only with respect to their localization in the organism but also with respect to their substrate specificity [1-3].

The substrate specificities of these enzymes have been studied with the use both of natural substrates and of synthetic analogs with different structures [3-5]. Specific substrates of ACE have been detected previously among synthetic analogs of acetylcholine [6, 7]: N-(β-acetoxyethyl)-N-methylmorpholinium and N-(β-acetoxyethyl)-N-methylpiperidinium. Out of the whole multiplicity of esters investigated, only acetyl-β-methylcholine is a specific substrate of ACE, and this is used as one of the main substrates in the study of the catalytic properties and identification of the cholinesterases [1, 8].

*There is considerable confusion in the original between alkyl and acyl radicals, and also in other respects, which it has been possible to resolve with certainty in only one or two cases — Translator.

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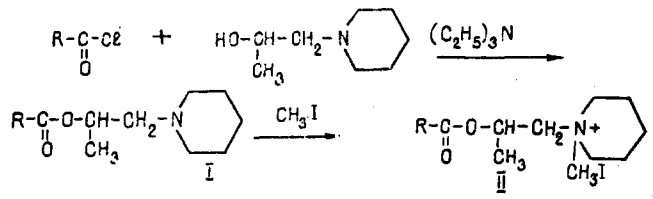
In order to find new substrates of cholinesterases we have synthesized (according to the scheme) a number of esters of N-(β-hydroxypropyl)piperidine analogous in their structure to acetyl-β-methylcholine.



In this paper we give the results of experimental investigations of the interactions of esters of N-(β-hydroxypropyl)pyridine (with alkyl radicals from CH_3 to C_4H_9) and their methiodides with the ACE from human blood erythrocytes and the BCE from horse blood serum.

The kinetic constants of the interaction of the esters based on piperidine with cholinesterases are given in Table 1, from which it follows that all the N-(β-acyloxypropyl)piperidines and their methiodides apart from N-(β-acetoxypropyl)piperidine methiodide inhibit the activities of both esterases reversibly (by the competitive type of inhibition).

Among the series of bases synthesized, the ethyl and butyl derivatives suppressed the enzymatic activity of ACE similarly. The hydrolysis of acetylthiocholine fell somewhat under the action of N-(β-isobutyloxypropyl)piperidine,* the value of the inhibition constant of which was three times lower than that of the substance with an ethyl radical and half that of compound with the normal butyl radical.

In the case of BCE, the highest antienzymatic activity was possessed by the compound having a propyl residue, the force of inactivation of which was more than eight times greater than the inhibitory properties of the methyl derivative. On the whole, the free N-(β-acyloxypropyl)piperidines inhibited the hydrolyzing capacity of BCE similarly.

The lengthening of the acyl radical from the acetate to the valerate led to a tenfold enhancement of the inhibiting efficiency for ACE and one of more than 30-fold for the BCE. This confirmed results obtained previously indicating that BCE has a broader hydrophobic section in the region of the esterase site than ACE.

In the methiodide series, the acetyl derivative at a concentration of $5 \cdot 10^{-2}$ M underwent slight hydrolysis under the action of ACE. At the same time, this compound reversibly inhibits the catalytic activity of BCE. A logical change in the structure of the acyl radical has an insignificant influence on the antienzymatic activity with respect to ACE. The preparation having a propyl radical in its molecule possessed an appreciable inhibiting activity in relation to BCE, the value of the inhibition constant (K_I) of which was more than 25 times greater than the antienzymatic properties of the acetyl derivative. This fact can only be explained by the assumption that BCE is more adapted to butyrylcholine and therefore reacts with the $C_3H_7-\overset{\overset{O}{\|}}{C}-O$ group of N-(β-butyryloxypropyl)piperidine,† which imitates the $C_3H_7-\overset{\overset{O}{\|}}{C}-S$ of butyrylthiocholine. A further increase in the size of the acyl residue does not lead to an appreciable antibutyrylcholinesterase activity. The iodomethylation of N-(β-hydroxypropyl)pyridine decreases its influence on the hydrolysis of acetylthiocholine as compared with butyrylthiocholine. The substance containing a propyl radical inhibits the hydrolyzing properties of both enzymes almost equally. As compared with its base, this compound is 17 times more effective for ACE and 5 times for BCE, which indicates a greater force of ion-ion interaction between the onium nitrogen heteroatom and the anionic site of the active surface of the esterases investigated.

Thus, a complication of the acyl moiety of the molecule of the compound synthesized, on the one hand, and also a replacement of the trimethyl group in the structure of acetyl-β-methylcholine by a piperidine ring, on the other hand, lead to a change in the type of inter-

*Judging from Table 1, N-(β-isovalenyloxypropyl)piperidine is meant — Translator.
†"N-(β-Propyloxypropyl)piperidine" according to the original — Translator.

TABLE 1. Kinetic Parameters of the Interaction of N-(β-Acyloxypropyl)piperidines and Their Methiodides with ACE and BCE

R	K_i (M)			
	I		II	
	ACE	BCE	ACE	BCE
CH_3	$6,4 \cdot 10^{-3}$	$3,2 \cdot 10^{-3}$	Is hydrolyzed at a concentration of $5 \cdot 10^{-2}$ M	$2,2 \cdot 10^{-3}$
C_2H_5	$5,0 \cdot 10^{-4}$	$1,0 \cdot 10^{-4}$	$1,5 \cdot 10^{-3}$	$6,0 \cdot 10^{-4}$
C_3H_7	$3,1 \cdot 10^{-3}$	$3,7 \cdot 10^{-4}$	$1,9 \cdot 10^{-4}$	$8,5 \cdot 10^{-5}$
C_4H_9	$6,6 \cdot 10^{-4}$	$1,0 \cdot 10^{-4}$	$3,4 \cdot 10^{-4}$	$2,2 \cdot 10^{-4}$
iso-C_4H_9	$1,5 \cdot 10^{-3}$	$2,0 \cdot 10^{-4}$	$8,6 \cdot 10^{-4}$	$6,0 \cdot 10^{-4}$

action with ACE and BCE: from substrate properties of the substance with an acetyl group (for ACE) to inhibitor properties of the other derivatives.

EXPERIMENTAL

Methods of Investigation. The catalytic activities of ACE (EC 3.1.1.7) and BCE (EC 3.1.1.8) were determined by Ellman's method [10] from the rate of hydrolysis of acetylthiocholine (ATC) for ACE and of butyrylthiocholine (BTC) for BCE. The accumulation of 5-mercapto-2-nitrobenzoic acid was recorded with the aid of a KFK-2 UKhL 4.2 photoelectric colorimeter at a wavelength of 400 nm. Esterase activities were determined at pH 7.5 and a temperature of 25°C.

The rate of the cholinesterase hydrolysis of N-(β-acetoxypropyl)piperidine was determined by the method of potentiometric titration [11]. The anticholinesterase activities of the piperidine derivatives were evaluated from the magnitude of the inhibited [sic] constant K_i found by the Lineweaver–Burk method [12].

Enzymes. The enzymes used were purified preparations of ACE (activity 3.5 units/mg) and BCE (activity 9.6 units/mg) produced by the Perm Scientific-Research Institute of Vaccines and Sera.

Substrates. Acetylcholine bromide, ATC, and BTC were Chemapol (Czechoslovakia) preparations. The 5,5'-dithio-bis-2-nitrobenzoic acid was a commercial preparation from Koch-Light.

SUMMARY

Of the ten esters of N-(β-hydroxypropyl)pyridine investigated, only the acetyl derivative is hydrolyzed under the action of ACE, and the remaining nine compounds inhibit the activities of ACE and BCE by the competitive type of inhibition, their inhibiting properties depending little on the size of the alkyl radical in the acid moiety of the inhibitor molecule.

LITERATURE CITED

1. A. P. Brestkin, I. L. Brick, and G. M. Grigor'eva, Int. Pharmacol. Ther. Sect., 85, 5, 241 (1973).
2. S. N. Golikov, and V. I. Rozengart, Cholinesterases and Anticholinesterase Agents [in Russian], Leningrad (1964).
3. A. S. Sadykov, E. V. Rozengart, A. A. Abduvakhabov, and Kh. A. Aslanov, Cholinesterases, Active Site and Action Mechanism [in Russian], Tashkent (1976).
4. A. P. Brestkin, E. V. Rozengart, I. N. Soboleva, N. V. Khromov-Borisov, and L. N. Tikhonova, Biokhimiya, 40, 95 (1975).
5. A. A. Abduvakhabov, A. A. Sadykov, D. N. Dalimov, and Kh. A. Aslanov, Alkaloids and Their Derivatives as Tools for the Study of the Cholinergic System [in Russian], Tashkent (1984).
6. E. V. Rozengart, I. N. Soboleva, N. L.Indenbom, L. N. Tikhonova, and N. V. Khromov-Borisov, Dokl. Akad. Nauk, SSSR, 209, 482 (1973).
7. Sh. K. Kasymov, A. A. Abduvakhabov, Kh. A. Aslanov, and E. V. Rozengart, Uzb. Khim. Zh., 3, 38 (1975).

8. V. I. Rozengart and O. E. Sherstobitov, The Selective Toxicity of Organophosphorus Insectoacaricides [in Russian], Leningrad (1978).
9. M. I. Kabachnik, A. A. Abduvakhabov, I. I. Agabekova, A. P. Brestkin, R. I. Volkova, N. N. Godovikov, E. I. Godyna, S. S. Mikhailov, M. Ya. Mikhel'son, V. I. Rozengart, E. V. Rozengart, and R. V. Sitkevich, Usp. Khim., 39, 1050 (1970).
10. G. L. Ellman, D. R. Cenntney, V. Anders, and K. M. Reather, Biochem. Pharm., 7, 88 (1961).
11. V. A. Yakovleb, The Kinetics of Enzymatic Catalysis [in Russian], Moscow (1965).
12. A. Cornish-Bowden, Principles of Enzyme Kinetics, Butterworths, London (1976).

IMMOBILIZATION OF THE ENZYME L-ASPARAGINASE FROM *E. coli* ON POLYSACCHARIDES

IV. COVALENT BINDING WITH DIALDEHYDE-DEXTRAN

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The synthesis has been effected of immobilized *E. coli* L-asparaginase on medical dextran — poliglyukin. The influence of the bound polymer on some physicochemical properties of the final products have been studied. An increased resistance to heat and stability on storage of the immobilized forms of L-asparagine in comparison with a native enzyme has been found. It has been shown that the polymer modification of L-asparaginase leads to a decrease in the antigenic affinity of the immobilized enzyme as compared with the native enzyme.

The most promising among the polymers used as supports for the immobilization of enzymes for medical purposes are dextrans, which is due to their high degree of biocompatibility and their utilization in the organism [1]. We have previously reported the immobilization of the antileukemia enzyme *E. coli* L-asparaginase on 3-bromo-2-hydroxypropyldextran (Sweden) [2]. In the present paper we give the results of the immobilization of L-asparaginase on a domestic dextran — poliglyukin. For direct binding of the enzyme we used poliglyukin previously oxidized by the periodate method to dialdehyde-poliglyukin. The labile azomethine bonds formed between the enzyme and the aldehyde groups of the polymer were reduced with sodium tetrahydroborate, which also lowered the excess of aldehyde groups in the poliglyukin.

A method has been described for obtaining L-asparaginase bound to dextrans by azide and cyanogen bromide methods [3, 4] using toxic reagents, which is impermissible in the creation of a medicinal preparation for intravenous purposes such as L-asparagine. The method given in the present paper avoids the use of toxic reagents. At the same time, the activation of poliglyukin is carried out in the production of streptodekaz. In the literature, this method has been described for the immobilization of *Erwinia carotovova* L-arparaginase [5-9]. At the same time, in clinics both in the USSR and abroad only *E. coli* L-asparaginase is widely used for treating diseases. The search for effective immobilized forms of the latter based on a biocompatible polymer used in medicine therefore acquires particular importance.

In the development of the optimum conditions for the synthesis of immobilized L-asparaginase it was established that the best results with respect to completeness of binding and yield of enzymatic activity are obtained at pH 8.5 and a temperature of 20 ± 2°C in the course of 3 h of synthesis at initial weight ratios of enzyme and poliglyukin of 1:2, 1:4, 1:6, and 1:8. The characteristics of the final products are given in Table 1.

The depth of binding of L-asparaginase with poliglyukin was estimated qualitatively by column gel chromatography on Sephadex G-200 and electrophoretically. As can be seen from Fig. 1, the rate of elution of the modified forms of L-asparaginase was higher than that of the native enzyme, which indicates an increase in the molecular weights of the final products.

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