EFFECT OF REVERSIBLE INHIBITORS ON THERMAL DENATURATION OF CHOLINESTERASES

V. D. Tonkopii and E. P. Padinker

UDC 577.152.311'1.043:536

The ability of reversible inhibitors (galanthamine and tacrine) to protect mouse and rat brain cholinesterase from thermal denaturation when the temperature is raised to 56 and 58°C was demonstrated. The protective action was exhibited when the reversible inhibitors lowered cholinesterase activity. The resistance of the cholinesterases to heat was greater when galanthamine was used than when tacrine was used.

KEY WORDS: brain cholinesterase; thermal denaturation; action of inhibitors.

Investigations have shown that certain ligands which interact with active centers of cholinesterase (CE), including acetylcholine and tetraethyl- and tetramethylammonium ions, increase the thermostability of the enzyme [1, 3].

This paper describes the results of a study of the effect of the reversible inhibitors galanthamine and tacrine on thermal denaturation of rat and mouse brain CE.

EXPERIMENTAL METHOD

Two series of experiments were carried out. In series I, rat brain homogenate was used as the source of CE. To prepare it, the brain (without the cerebellum) was removed and homogenized in a Teflon–glass homogenizer with physiological saline in the ratio of 1:19. To 1 ml homogenate 1 ml of aqueous solutions of the reversible inhibitors was added in appropriate concentrations and the mixture was incubated at 58°C for 20 min. In parallel experiments by Hestrin's method [5] the anticholinesterase activity of the preparations was determined on incubation with the homogenates at 20°C. Acetylcholine iodide was used as the substrate.

In the experiments of series II, galanthamine and tacrine were injected intraperitoneally into the mice in a dose of 4 mg/kg. The animals were killed 30, 60, 120, and 180 min later; the brain was removed (with-

TABLE 1. Protection of Rat Brain CE against Thermal Denaturation in Presence of Galanthamine and Tacrine in Various Concentrations (M ± m)

Concentration of preparations (M)	Inhibition of CE activity (% of normal)			
	galanthamine		tacrine	
	at 20°	at 58°	at 20°	at 58°
—	—	90,0±1,7	—	90±1,7
$1{,}25 \cdot 10^{-5}$	33,0±2,1	24,0±3,8	—	—
$2{,}5 \cdot 10^{-6}$	18,0±1,7	45,0±4,2	—	—
$1{,}25 \cdot 10^{-6}$	16,0±1,2	56,0±2,9	60,0±1,8	24,0±0,9
$5 \cdot 10^{-7}$	12,0±2,1	60,0±4,1	42,0±2,3	30,0±1,5
$2{,}5 \cdot 10^{-7}$	10,0±0,9	72,0±3,7	34,0±1,7	42,0±3,2
$5 \cdot 10^{-8}$	0	91,0±2,5	19,0±2,5	63,0±2,3
$2{,}5 \cdot 10^{-8}$	—	—	9,0±1,2	82,0±4,2

S. M. Kirov Military Medical Academy, Leningrad. (Presented by Academician of the Academy of Medical Sciences of the USSR S. N. Golikov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 79, No. 4, pp. 51-52, April, 1975. Original article submitted April 22, 1974.

TABLE 2. Protection of Mouse Brain CE against Thermal Denaturation at Various Times after Injection of Galanthamine and Tacrine in a Dose of 4 mg/kg (M ± m)

Time after injection of preparations (min)	Inhibition of CE activity (% of normal)			
	galanthamine		tacrine	
	true inhibition	at 56°	true inhibition	at 56°
Control	—	80,0±1,8	—	80,0±1,8
30	77,5±2,1	52,8±1,7	70,0±2,8	62,0±2,6
60	62,0±1,8	56,0±2,3	47,5±2,1	73,5±3,1
120	53,0±2,3	60,0±3,2	24,0±1,3	82,0±2,2
180	20,3±1,7	67,0±1,8	0	80,0±2,7

out the cerebellum) and placed intact in test tubes containing physiological saline heated on a water bath at 56°C for 20 min. The residual enzyme activity was then determined in the homogenates. The degree of inhibition of brain CE activity in the animals after injection of the preparations also was determined by a special method designed to assess the true inhibition of CE activity after treatment with reversible inhibitors [2].

EXPERIMENTAL RESULTS

Elevation of the temperature led to inhibition of the rat brain CE activity by 90.0 ± 1.7 % and mouse brain CE activity by 80.0 ± 1.8 %. Preliminary incubation of the enzyme with the reversible inhibitors was followed by a much lesser degree of denaturation of CE and preservation of its activity (Table 1). Galanthamine was better able to protect the rat brain enzyme than tacrine. For instance, with identical inhibition of CE by the reversible inhibitors (by 33-34%) the increase in inhibition caused by thermal denaturation was 24.0 ± 3.8 % in the presence of galanthamine but 42.0 ± 3.2 % in the presence of tacrine ($P < 0.001$). A similar pattern was found when the effect of the denaturing factor on mouse brain CE was studied. Inhibition of brain CE activity 30 min after injection of the preparations was almost identical, but the resistance of the enzyme to heat was greater after galanthamine (Table 2). The increase in thermostability of the CE coincided in time with the inhibition of enzyme activity induced by the inhibitors.

Protein denaturation is accompanied by structural changes in the protein molecule involving a disturbance of its secondary and tertiary structures [4]. Interaction between reversible inhibitors and CE creates a more stable and orderly structure and stabilizes the enzyme. This is evidently the reason for the increased thermostability of the reversible inhibitor–enzyme complex.

LITERATURE CITED

1. G. M. Grigor'eva, "The role of the anionic center of the active surface of cholinesterases," Author's Abstract of Candidate's Dissertation, Leningrad (1965).
2. V. D. Tonkopii, N. V. Savateev, A. P. Brestkin, et al., Dokl. Akad. Nauk SSSR, 207, No. 3, 736 (1972).
3. M. H. Coleman and D. D. Eley, Biochim. Biophys. Acta, 67, 646 (1963).
4. M. H. D. Joly, A Physico-Chemical Approach to the Denaturation of Protein, Academic Press (1965).
5. S. Hestrin, J. Biol. Chem., 180, 249 (1949).