PROTECTIVE EFFECT OF 1,2,3,4-TETRAHYDRO-9-AMINOACRIDINE ON ACETYLCHOLINESTERASE INHIBITION BY ORGANOPHOSPHORUS INHIBITORS

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Received May 15th, 1975

It has been demonstrated that 1,2,3,4-tetrahydro-9-aminoacridine inhibits acetylcholinesterase and simultaneously alters its affinity for organophosphorus inhibitors. The coupling of the inhibitor to the enzyme decreases its affinity for organophosphates; hence the inhibitor has virtually a protective effect. The change in affinity is most likely caused by conformational changes of the acetylcholinesterase molecule.

It has been observed earlier[1,2] that tetrahydro-9-aminoacridine (THA) acts as a non-competitive inhibitor of acetylcholinesterase with a K_i of 2 μM. THA binds to the gamma-anionic site of the active surface of the enzyme through a hydrophobic bond and forms an irreversible complex of one inhibitor molecule with one active center. The coupling of THA to acetylcholinesterase brings about conformational changes in the enzyme molecule[2,3], as evidenced in this paper reporting on the effect of THA on the inhibition of acetylcholinesterase by certain organophosphorus inhibitors *in vitro*.

EXPERIMENTAL

Chemicals. Tetrahydro-9-aminoacridine hydrochloride was prepared in the form of a colorless crystalline product, m.p. 284—287°C, recorded[4] m.p. 283—284°C. The compound was identified by elemental analysis and IR-spectra measurement. O-Isopropylmethylfluorophosphonate (*I*), O-pinacolylmethylfluorophosphonate (*II*), O-ethyl-S-(2-dimethylaminoethyl)methylthiophosphonate (*III*) and O-ethyl-S-(2-diisopropylaminoethyl)methylthiophosphonate (*IV*) were generous gifts of Dr. J. Vachek of this Institute. The remaining chemicals of analytical purity were from Lachema, Brno.

Enzyme. Homogenates of whole rat brains served as a source of the enzyme. Wistar rats (200 to 400 g) of both sexes were sacrificed by scission of the carotids and bleeding. The brains were rapidly excised and residual blood washed off with cold physiological saline. The brain tissue was homogenized in 0·9% NaCl in an Ultra Turrax blender to a 10% homogenate. The enzyme activity of the homogenate varied between 0·5 and 0·6 μmol of degraded acetylcholine . min^{-1} . . ml^{-1} at 25°C and pH 8·0.

Measurement of enzymatic activity. The activity of acetylcholinesterase was measured as initial rate of acetylcholine hydrolysis at final concentration 5 mM in a Radiometer pH-stat as described elsewhere[2]. The measurements were made at 25°C and pH 8·0. The inhibition experiments were allowed to proceed as follows. The enzyme (0·5 ml) was incubated 30 min with the organophosphate solution of known concentration, buffered physiological saline (0·005M barbital phosphate, pH 8·0) containing the substrate was added afterwards to make up the volume of the mixture to 20 ml, and residual enzyme activity was measured afterwards. In experiments with the protective effect of THA the enzyme was incubated 10 min with 2, 4, 6, 10 or 20 μM of THA and then 30 min with the organophosphorus inhibitor. All inhibition experiments were carried out at 25°C and pH 8·0. The inhibitor concentration causing a 50% inhibition (I_{50}) and its negative decadic logarithm (pI_{50}) were calculated from the plot of per cent of inhibition *versus* organophosphorus inhibitor concentration by the probit-logarithmic transformation. These constants were calculated on MINSK-22 computer. The magnitudes of the constants given are means ± confidence interval for P 0·95.

RESULTS AND DISCUSSION

The incubation of acetylcholinesterase with THA leads to an inhibition of the enzyme activity and to a change of its affinity for organophosphorus inhibitors. The change in affinity manifests itself by a shift of the inhibition curves toward higher inhibitor concentrations, as demonstrated for O-ethyl-S-(2-diisopropylaminoethyl)methyl-

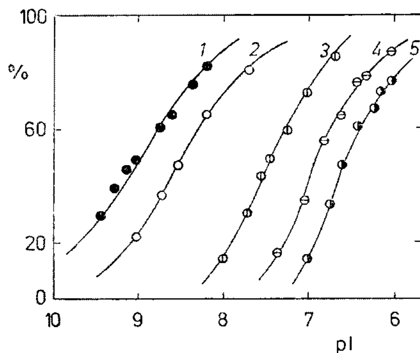

FIG. 1

Plot of Per Cent of Inhibition of Tetrahydro-9-aminoacridine-modified Acetylcholinesterase on Concentration of O-ethyl-S-(2-diisopropylaminoethyl)methylthiophosphonate

Acetylcholinesterase was preincubated 10 min with tetrahydro-9-aminoacridine: 0 μM, *1* 2 μM, *2* 4 μM, *3* 6 μM, *4* and 10 μM, *5*, then 30 min with the organophosphorus inhibitor. Each point represents a mean value of two measurements.

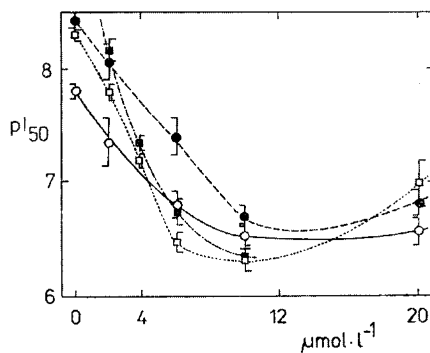

FIG. 2

Plot of Affinity of Organophosphorus Inhibitors for Acetylcholinesterase (pI_{50}) *versus* Tetrahydro-9-aminoacridine Concentration

The enzyme was preincubated 10 min with THA (μmol . l^{-1}). Compound *I* (○), compound *II* (●), compound *III* (□), and compound *IV* (■). The bars represent the confidence interval for P 0·95.

thiophosphonate in Fig. 1; there are no significant changes in the slope or shape of the inhibition curves. The plot of the logarithm of affinity constants (pI_{50}) expressing the affinity of organophosphates for acetylcholinesterase *versus* THA concentration, with which the enzyme was preincubated, is shown in Fig. 2. The I_{50}-value together with the ratio of these constants characterizing the enzyme treated and not treated (P) with THA are summarized in Table I. The preincubation of acetylcholinesterase with individual concentrations of THA causes the following degree of inhibition: 2 μM 46, 4 μM 58, 6 μM 70, 10 μM 80, and 20 μM 87%.

TABLE I

Affinity of Organophosphorus Inhibitors for Acetylcholinesterase Modified by 1,2,3,4-Tetrahydro-9-aminoacridine

I_{50} is the concentration of organophosphorus inhibitor which causes a 50% inhibition after 30 min incubation with the enzyme. The lower and upper limit of the confidence interval for P 0·95 are given in brackets. P indicates the I^*_{50}/I_{50} ratio, where I^*_{50} is the affinity constant measured with the THA-treated enzyme and I_{50} the affinity constant measured with the unmodified enzyme.

Organophosphorus compound	[THA] μM	I_{50}	P
I	0	1·55 (1·36—1·77) . 10^{-8}	—
	2	4·67 (2·71—8·04) . 10^{-8}	3·0
	6	1·67 (1·30—2·15) . 10^{-7}	10·8
	10	3·10 (2·49—3·63) . 10^{-7}	20·0
	20	2·62 (1·84—3·72) . 10^{-7}	17·0
II	0	3·60 (3·15—4·10) . 10^{-9}	—
	2	8·64 (6·16—12·11) . 10^{-9}	2·4
	6	3·99 (2·73—5·84) . 10^{-8}	11·1
	10	2·02 (1·57—2·59) . 10^{-7}	66·0
	20	1·62 (1·27—2·07) . 10^{-7}	45·0
III	0	4·95 (4·29—5·70) . 10^{-9}	—
	2	1·55 (1·29—1·85) . 10^{-8}	3·1
	4	3·62 (3·10—4·21) . 10^{-8}	7·3
	6	3·28 (2·75—3·89) . 10^{-7}	66·0
	10	4·56 (3·57—5·87) . 10^{-7}	92·0
	20	1·09 (0·71—1·68) . 10^{-7}	22·0
IV	0	1·08 (0·82—1·43) . 10^{-9}	—
	2	6·70 (5·71—7·86) . 10^{-9}	6·2
	4	4·62 (3·93—5·43) . 10^{-8}	42·7
	6	1·94 (1·56—2·42) . 10^{-7}	179·0
	10	4·02 (3·58—4·51) . 10^{-7}	372·0

Tetrahydro-9-aminoacridine binds to the hydrophobic domain of the active surface of acetylcholinesterase[2] simultaneously affecting its catalytic center *via* an allosteric mechanism shown to exist with this enzyme by other authors earlier[5-7]. The form of this influence of THA is probably that of a conformational change of the enzyme molecule and manifests itself among others by a change in the affinity of the catalytic center for organophosphorus inhibitors, as has been demonstrated in the case of O-isopropyl-methylfluorophosphonate first by Heilbronn[1]. The affinity of organophosphates for THA-modified acetylcholinesterase decreases with the increasing concentration of THA up to 10 μM concentration. The protective effect is maximum at this concentration for all the organophosphates examined. When the protective effect is expressed by index $P = I^*_{50}/I_{50}$, where I^*_{50} is the value measured with THA-modified acetylcholinesterase, the lowest protective effect shows compound *I* and the highest compound *IV*. The following values of P were obtained at 10 μM concentration of THA: compound *I* 20, compound *II* 66, compound *III* 92, and compound *IV* 372; these values correspond to the following changes in free energy: 1·78, 2·48, 2·67, and 3·51 kcal mol^{-1} (calculated from the formula[8] $\Delta\,\Delta F = 2{\cdot}303RT\,.\,\log(I^*_{50}/I_{50})$, modified for 25°C to $\Delta\,\Delta F = 1{\cdot}364 \log P$).

The $\Delta\,\Delta F$-value increases with the total volume of the organophosphorus inhibitor molecule and is in relation to the calculated values of molar refraction R_0 whose value bears relation to the volume of the molecule. There is a linear relation between both magnitudes, $R_0 = -11{\cdot}89 + 24{\cdot}90\Delta\,\Delta F$, the correlation coefficient being r_{xy} 0·989; the correlation is therefore significant for $P > 0{\cdot}99$.

The authors thank Mrs V. Pacovská for the computer calculations and Mr O. Ochrymovič for technical assistance.

REFERENCES

1. Heilbronn E.: Acta Chem. Scand. *15*, 1386 (1961).
2. Patočka J., Bajgar J., Bielavský J., Fusek J.: This Journal *41*, 816 (1976).
3. Fusek J., Patočka J., Bajgar J., Bielavský J., Herink J., Hrdina V.: Activ. Nerv. Super. *16*. 226 (1974).
4. Petrow V.: J. Chem. Soc. *634* (1947).
5. Kitz R. J., Braswell L. M., Ginsburg S.: Mol. Pharmacol. *6*, 108 (1970).
6. Roufogalis B. D., Quist E. E.: Mol. Pharmacol. *8*, 41 (1972).
7. Kato G., Tan E., Yung J.: Nature New Biol. *236*, 185 (1972).
8. Webb J. L.: Enzym. Metab. Inhib. *1*, 269 (1963).

Translated by V. Kostka.