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AGING OF SOMAN-INHIBITED ACETYLCHOLINESTERASE: INHIBITORS AND ACCELERATORS

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Summary

The influence of 27 possible effectors, mostly bispyridinium salts, upon the dealkylation (aging) of soman-inhibited acetylcholinesterase (acetylcholine hydrolase, EC 3.1.1.7) was examined at pH 7.6 and 25°C. In the absence of effectors, the rate constant of the aging process was $4.0 \cdot 10^{-2}$ min^{-1}. At 2 mM, the strongest inhibitor reduced the rate to $0.8 \cdot 10^{-2}$ min^{-1}, whereas it was raised to $8.2 \cdot 10^{-2}$ min^{-1} by the most potent accelerator.

Acetylcholinesterase (acetylcholine hydrolase, EC 3.1.1.7), phosphonylated by soman (*O*-(1,2,2-trimethylpropyl)methylphosphonofluoridate), undergoes rapid dealkylation by loss of the 1,2,2-trimethylpropyl residue. This reaction is called aging. The aged fraction of the inhibited enzyme cannot be reactivated by acetylcholinesterase-reactivators such as pyridine-2-aldoxime methoiodide (2-PAM) [1].

The aging reaction proceeds relatively fast in vitro as well as in vivo, so that up to now, no satisfactory therapeutic measures against intoxications by soman are known. On the other hand, the inability of 2-PAM to reactivate the dealkylated phosphonyl-acetylcholinesterase can be used to determine the fraction of aged enzyme and thus to follow the time course of the aging reaction.

With regard to the therapeutic aspect as well as to the question of the reaction mechanism, it is of some interest to study the conditions by which the aging rate can be influenced. In general, pH increase, increase in ionic strength and lowering of the temperature decreases the aging velocity [1]; furthermore, certain ammonium salts and amines were found to retard aging [1—5].
In a previous paper we described the retardation of aging caused by some mono- and bispyridinium salts, the latter being the more effective compounds [6]. In continuing these studies we tested some other bispyridinium salts and

non-quaternary compounds upon their ability to influence aging.

The compounds HH 38, HH 39, HH 54 [7] and the series from P 60 to P 66 [8] were synthesized according to the cited literature, SAD 128 was prepared by Dr. R. Reiner, Frankfurt. All other substances were gifts from Prof. I. Hagedorn, Freiburg/Br. The acetylcholinesterase from bovine erythrocytes (Serva, Heidelberg) contained $5.5 \cdot 10^{-12}$ mol active sites per mg, as determined by "titration" with soman [9].

To a solution of 10 mg ($5.5 \cdot 10^{-11}$ mol) acetylcholinesterase in 0.5 ml 0.5 mM Tris/HCl buffer (pH 8.8, 1° C), $11 \cdot 10^{-11}$ mol soman (5 μl of a stock solution in absolute ethanol) were added. One half of the soman, i.e. an amount equivalent to the acetylcholinesterase present, is considered to consist of the two fast-reacting isomers [1]. The mixture was kept at 1° C for 30 min. The residual activity (less than 3%) was controlled using a 20-μl sample. During the 30 min incubation period the phosphonylated enzyme underwent aging by about 5%.

To start the aging process, the test tube containing the inhibited enzyme was transferred into a water bath controlled at 25° C and 0.5 ml 0.1 M Tris/HCl buffer (pH 7.6) were added. The compounds to be tested were included into this buffer volume. The final salt concentrations in the reaction mixture were: 0.05 M Tris/HCl, 0.1 M NaCl, 0.02 M $MgCl_2$. At appropriate time intervals 0.1-ml samples were added to 0.1 ml 10 mM 2-PAM solution in 50 mM Tris/HCl buffer (pH 7.6, 25° C).

After 90 min incubation, when maximal reactivation was attained, 0.1 ml were transferred into the reaction vessel of a Metrohm autotitrator containing 5 ml 0.155 M NaCl. After addition of acetylcholine chloride (final concentration 27 mM) the enzymatic activity was determined by pH-stat titration at pH 7.0 and 25° C. The measured activity was corrected for the inhibitory effect of 2-PAM and the respective test compound, which had been determined before in a separate assay.

The first-order rate constants of aging k_a were calculated from the decrease of maximal reactivatability vs. time (Table I). The rate constant of aging in absence of any effector was determined under the same conditions to be $k_{a_0} = 4.0 \cdot 10^{-2}$ min^{-1} (S.D. 2%).

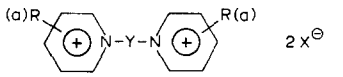
The data for the series from P 60 to P 66 including the results for the following four compounds show that the length of the bridge between the two pyridinium moieties is of minor importance for the retarding effect. The highest retarding efficiencies were found with the urea-bridged compounds HH 38 and HH 54. In this group the data show interesting structure-activity relations. From the few examples given here it becomes obvious, that the retarding effect depends upon the concentration and that for different effectors in a different degree.

The semicarbazones HP 70 and HP 71 accelerate the aging reaction. This effect was also observed with semicarbazide itself but surprisingly not with the semicarbazone from acetone. The results with urea and thiourea suggest that the hydrazide group seems to be essential for this special activity. Perhaps compounds of this kind can serve as useful tools for further investigations on the mechanism of aging.

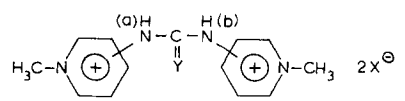
As reported in an earlier paper [8], some of the compounds tested here exhibit remarkable affinities towards the nonphosphonylated acetylcholinesterase. It seems worth mentioning that there is no correlation between these data and their abilities to influence the aging process of the phosphonylated enzyme.

TABLE I

Rate constants of aging of soman-inhibited acetylcholinesterase in presence of different effectors, as determined at 25°C, pH 7.6 in 0.05 M Tris/HCl buffer, 0.1 M NaCl, 0.02 M $MgCl_2$. Aging rate in absence of effector $k_{a_0} = 4.0 \cdot 10^{-2}$ min^{-1} (S.D. ± 2%). The data are mean values from at least three experiments, the S.D. being ⩽ 10%.



Effector	a	R	Y	X	$k_a \cdot 10^{-2}$ (min^{-1}) at effector concn.		
					0.5 mM	1 mM	2 mM
P 60	4	$-C(CH_3)_3$	$-CH_2-$	Br			3.4
P 61	4	$-C(CH_3)_3$	$-(CH_2)_2-$	Br			2.3
P 62	4	$-C(CH_3)_3$	$-(CH_2)_3-$	I			2.2
P 63	4	$-C(CH_3)_3$	$-(CH_2)_4-$	Br			2.0
P 64	4	$-C(CH_3)_3$	$-(CH_2)_5-$	Br			2.1
P 65	4	$-C(CH_3)_3$	$-(CH_2)_6-$	Br	2.4		2.1
P 66	4	$-C(CH_3)_3$	$-(CH_2)_7-$	Br	2.7		2.1
SAD 128	4	$-C(CH_3)_3$	$-CH_2-O-CH_2-$	Cl			2.1
HC 12	4	$-C(CH_3)_3$	$-O-(CH_2)_2-O-$	Cl			2.1
HY 10	4	$-CH=NO-C(CH_3)_3$	$-CH_2-O-CH_2-$	I	4.0	3.0	1.9
HY 21	4	$-CONH-C(CH_3)_3$	$-CH_2-O-CH_2-$	I		3.3	2.0
HH 20	3	$-NHCO-NH_2$	$-CH_2-O-CH_2-$	Cl			2.1
HP 73	3	$-NHCO-CH_3$	$-CH_2-O-CH_2-$	Cl			2.1
HP 70	3	$-CH=NNHCO-NH_2$	$-CH_2-O-CH_2-$	Cl			5.1
HP 71	4	$-CH=NNHCO-NH_2$	$-CH_2-O-CH_2-$	Cl			8.2



Effector	a	b	Y	X	$k_a \cdot 10^2$ (min^{-1}) at effector concentration		
					0.5 mM	1 mM	2 mM
HH 38	3	3	=O	I	1.8	1.3	0.8
HH 39	4	4	=O	I	2.9		2.4
HH 54	3	4	=O	I	1.5	1.3	1.1
HH 65	3	3	=S	I			2.8
HH 60	4	4	$=H_2$	I			1.6
HH 69	3	3	=NH	I			2.8

Other compounds	Formula	$k_a \cdot 10^{-2}$ (min^{-1}) at a concentration of 2 mM
Acetonsemicarbazone	$(H_3C)_2C=NNHCONH_2$	4.0
Semicarbazide	$H_2NNHCONH_2$	5.8
Urea	H_2NCONH_2	3.6
Thiourea	H_2NCSNH_2	3.5
NaF		3.4
Acetylcholine		3.9

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