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Supplementary Material Available: The X-ray crystallographic determination of 1·HCl, listings of fractional atomic coordinates with their estimated standard deviations, temperature factors, intramolecular distances and angles, least-squares planes, and observed and calculated structure factors, and force field calculations giving complete coordinates for structures A–D (13 pages). Ordering information is given on any current masthead page.

Effects of Charge, Volume, and Surface on Binding of Inhibitor and Substrate Moieties to Acetylcholinesterase

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Reversible inhibitors for acetylcholinesterase, AcChE, have been studied. Sterically similar alcohols with tetrasubstituted uncharged β groups, $(CH_3)_3SiCH_2CH_2OH$ (I), $(CH_3)_3CCH_2CH_2OH$ (IA), and $CH_3S(O_2)CH_2CH_2OH$ (VII), bind similarly, K_I = 3–9 mM, and each binds similarly to its acetate substrate; cationic analogues, $(CH_3)_3N^+CH_2CH_2OH$ (IB) and $(CH_3)_2S^+CH_2CH_2OH$ (II), bind similarly to each other, K_I = 0.4 mM, similar to K_m values of their acetate substrates, and more strongly than the uncharged alcohols by ~1.5 kcal/mol. In comparisons of VII with $CH_3SO_2CH_3$, II with $(CH_3)_3S^+$, and IB with $(CH_3)_4N^+$, hydroxyethyl leads to more favorable binding than methyl by ~0.8 kcal/mol, despite lower hydrophobicity. Two hydrophobic methyl groups, in comparison of IA with butanol, and two hydrophilic sulfone O atoms, in comparison of VII with 2-(methylthio)ethanol, increase binding similarly, by 1.0 kcal/mol. Conversion of $(CH_3)_3S^+$ to $(CH_3)_3S^+O$ also improves binding. However, $(CH_3)_3N^+O^-$ does not bind to AcChE, and conversion of 1-(dimethylammonio)-4-pentanone and 2-(dimethylammonio)ethyl acetate to their *N*-oxides, changes of $\equiv N^+H$ to $\equiv N^+—O^-$, decreases binding by 1.5 kcal/mol. Although the $-COCH_3$ group in esters with well-binding β substituents makes essentially no contribution to binding over that of the alcohols, in esters with weakly bound β substituents, $(CH_3)_2N^+(O^-)$, $CH_3N^+H_2$, $CH_3S(O)$, CH_3CH_2, and CH_3S binding is dominated by the ester $-COCH_3$ group, with values of K_m ~ 16 mM.

Acetylcholinesterase, AcChE, hydrolyzes ethyl acetates, $X-CH_2CH_2OCOCH_3$, with cationic,[1] nonpolar,[2] and uncharged polar[3] β substituents, X, of varied structure. Enzymic reactivity normalized for effect of β substituents on intrinsic alkaline hydrolytic reactivity, $(k_{cat}/K_m)_n$, for cationic and neutral substrates with X = $(CH_3)_3C$, $(CH_3)_3N^+$, $(CH_3)_2CH$, $(CH_3)_2S^+$, CH_3CH_2, Br, Cl and H was correlated with calculated refraction volumes, MR, while with X = CH_3S, $CH_3S(O)$, $(CH_3)_2N^+O^-$ and $CH_3S(O_2)$ reactivity was *lower* than consistent with MR by factors of 5–40.[4] Normalized reactivity of substrates with β substituents Cl, Br, CH_3S, CH_3CH_2, $(CH_3)_2CH$, $(CH_3)_3C$, and $(CH_3)_3Si$ correlated with hydrophobicity, π, but the cationic and dipolar substituents, $(CH_3)_3N^+$, $(CH_3)_2S^+$, $CH_3S(O_2)$, $CH_3S(O)$, and $(CH_3)_2N^+(O)^-$, led to reactivity greater than consistent with a relation to π by factors of 7–400, with the cationic substituents showing the greatest discrepancies.[4] Thus, it appeared that there is a more general and relevant correlation of reactivity with volume than with hydrophobicity, π, i.e. favorable lipid to water solubility ratio, and that maximum reactivity, correlated with volume, may depend on presence of a hydrophobic surface.[4] This was consistent with the view that the binding subsite for the β substituent may be termed *trimethyl* rather than *anionic*, apparently complementary to the hydrocarbon surface of analogous cationic and uncharged branched β substituents.[5] Support for the uncharged character of this subsite was seen in the equal effectiveness of α-bromopinacolone, $(CH_3)_3CCOCH_2Br$, in irreversible inhibition of hydrolysis of acetylcholine and its uncharged carbon analogue, 3,3-dimethylbutyl acetate.[6]

Cationic charge increases binding of cationic as compared with isosteric uncharged reversible inhibitors structurally related to acetylcholine, by about a factor of 10,[7] as the isoelectric point of the enzyme, ~5,[8] leads to multiple nonspecific anionic charges in the region of the active site[9] at the higher pH, 7–8, at which the enzyme acts. That these sterically similar cationic and uncharged pairs inhibit acetylcholine and its uncharged analogue equally indicates that a single subsite is involved in the binding

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(1) Wilson, I. B.; Cabib, E. *J. Am. Chem. Soc.* **1956**, *78*, 202–207.
(2) Adams, D. H. *Biochim. Biophys. Acta* **1949**, *3*, 1–14.
(3) Jarv, J.; Kesvatera, T.; Aaviksaar, A. *Eur. J. Biochem.* **1976**, *67*, 315–322.
(4) Cohen, S. G.; Elkind, J. L.; Chishti, S. B.; Giner, J. L.; Reese, H.; Cohen, J. B. *J. Med. Chem.* **1984**, *27*, 1643–1647.
(5) Hasan, F. B.; Cohen, S. G.; Cohen, J. B. *J. Biol. Chem.* **1980**, *255*, 3898–3904.
(6) Cohen, S. G.; Lieberman, D. L.; Hasan, F. B.; Cohen, J. B. *J. Biol. Chem.* **1982**, *257*, 14087–14092.
(7) Hasan, F. B.; Elkind, J. L.; Cohen, S. G.; Cohen, J. B. *J. Biol. Chem.* **1981**, *256*, 7781–7785.
(8) Leuzinger, W.; Baker, A. L.; Cauvin, E. *Proc. Natl. Acad. Sci. U.S.A.* **1968**, *59*, 620–623.
(9) Nolte, H.-J.; Rosenberry, T. L.; Neumann, E. *Biochemistry* **1980**, *19*, 3705–3711.

0022-2623/85/1828-1309$01.50/0

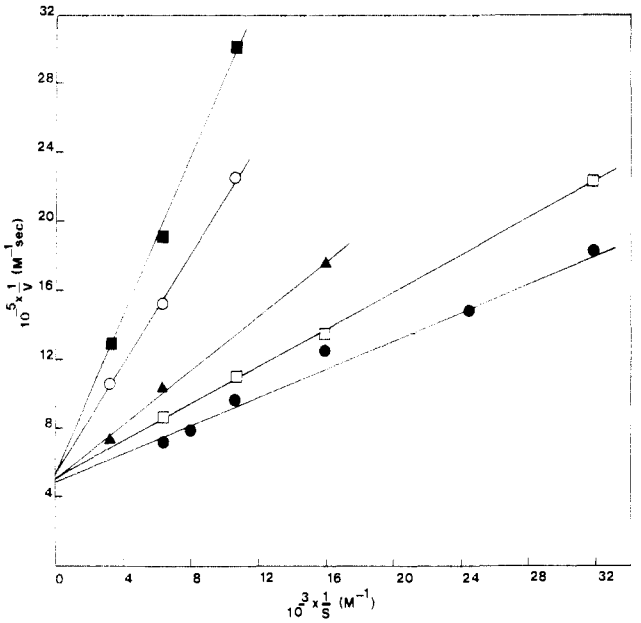

Figure 1. Inhibition by $(CH_3)_3SiCH_2CH_2OH$ (I) of hydrolysis of AcCh by AcChE: ●, no inhibitor; □, 1.81 mM I; ▲, 4.52 mM I; ○, 11.9 mM I; ■, 17.9 mM I.

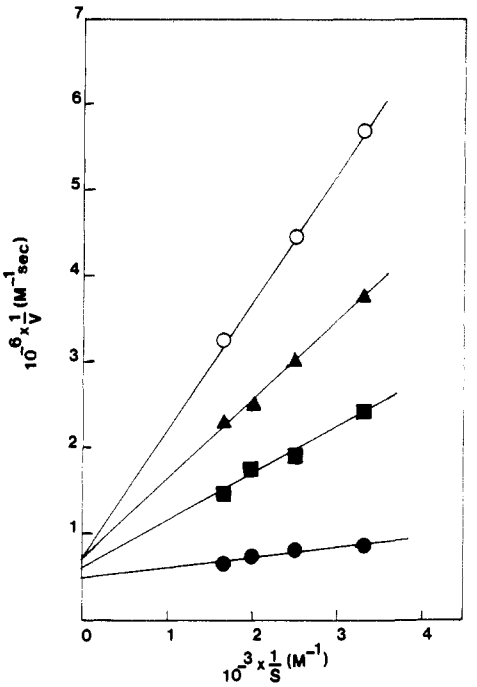

Figure 2. Inhibition by $(CH_3)_2S^+CH_2CH_2OHI^-$ (II): ●, no inhibitor; ■, 2.25 mM II; ▲, 4.5 mM II; ○, 6.75 mM II.

of these compounds.[7] We now report on a study of reversible inhibitors with β substituents of varying surface atom character, i.e. C–H and O, and core atom size and charge, N^+, C, S, S^+, and Si, and compare their binding with that of structurally related substrates[4] that more completely occupy the active site.

Results

The inhibitors to be described comprise ethanols with branched and linear β substituents, $X\text{–}CH_2CH_2OH$, X = $(CH_3)_3Si$, $(CH_3)_3C$, $(CH_3)_3N^+$, $(CH_3)_2S^+$, $CH_3S(O_2)$, CH_3S, and CH_3CH_2, compounds related to certain of these, with methyl replacing hydroxyethyl, trimethylsulfonium ion $(CH_3)_3S^+$, its oxide, $(CH_3)_3S^+(O)$, dimethylsulfone, $(CH_3)_2S(O_2)$, and dimethyl sulfoxide $(CH_3)_2SO$, trimethylamine oxide, $(CH_3)_3N^+O^-$, and a related ketone sterically

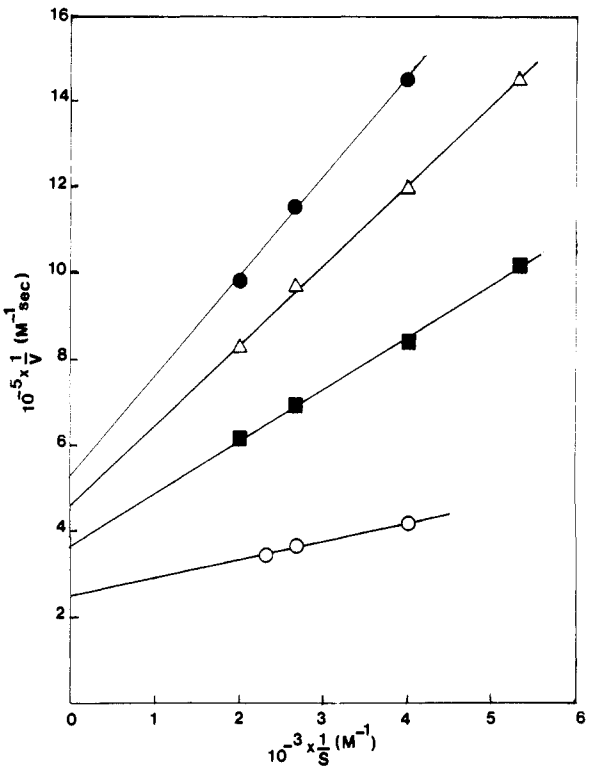

Figure 3. Inhibition by $(CH_3)_3S^+I^-$ (III): ○, no inhibitor; ■, 2.47 mM III; △, 4.93 mM III; ●, 7.39 mM III.

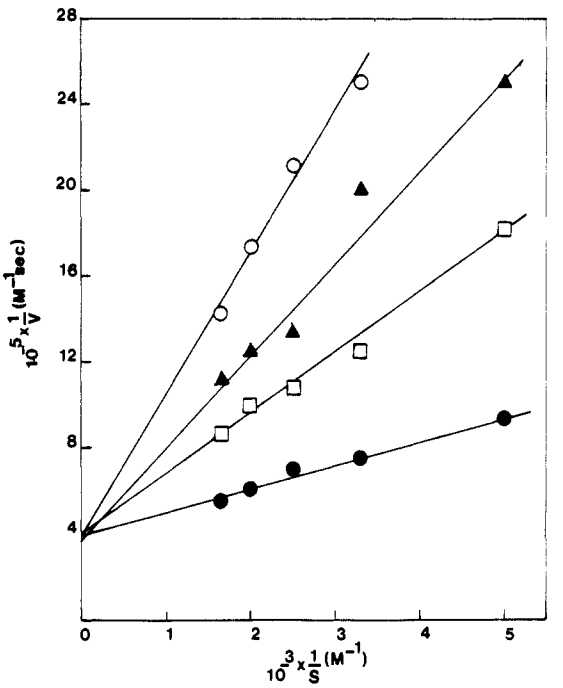

Figure 4. Inhibition by $(CH_3)_3S^+OI^-$ (IV): ●, no inhibitor; □, 2.25 mM IV; ▲, 4.5 mM IV; ○, 6.75 mM IV.

similar to acetylcholine, $(CH_3)_2N^+(O^-)CH_2CH_2CH_2COCH_3$.

Plots of $1/V$ vs. $1/S$ for the more effective inhibitors that have not been previously reported, compounds I–IV and VII (Table I), are given in Figures 1–5. Least-squares slopes and intercepts (i) for all the $1/V$ vs. $1/S$ data, (ii) for these slopes against inhibitor concentrations, and (iii) for the intercepts of these reciprocal plots for compounds II, III, VII, and VIIA against inhibitor concentrations are given under Materials and Methods. Ratios of intercept to slope of the secondary slope vs. inhibitor concentration plots gave values of competitive $K_{I(com)}$; these ratios of the secondary intercept vs. inhibitor concentration plots gave

Table I. Reversible Inhibition of Hydrolysis of Acetylcholine by Acetylcholinesterase (pH 7.8, 25 °C, 0.18 M NaCl)

no.	compd	$K_{I(com)}$,[a] mM	$K_{I(nonc)}$,[a] mM	K_m,[b] mM	MR_β,[c] cm^3
I	$(CH_3)_3SiCH_2CH_2OH$	3.3		3.5	25.0
IA	$(CH_3)_3CCH_2CH_2OH$	7.5[d]	19[d]	5.3	19.6
IB	$(CH_3)_4N^+CH_2CH_2OHCl^-$	0.4[e]	7.6[e]	0.33	17.2
II	$(CH_3)_2S^+CH_2CH_2OHI^-$	0.4	13	0.33	16.4
III	$(CH_3)_3S^+I^-$	2.0	7.2		
IV	$(CH_3)_3S^+OI^-$	1.3			
V	$(CH_3)_3N^+O^-$	≫200		18	
VI	$(CH_3)_2N^+(O^-)CH_2CH_2CH_2COCH_3$	14		18	16.4
VII	$CH_3S(O_2)CH_2CH_2OH$	8.7	100[f]	6.2	13.9
VIIA	$CH_3S(O_2)CH_2CH_2OCOCH_3$	6.4	11	6.2	
VIII	$(CH_3)_2SO_2$	28	260		
IX	$(CH_3)_2SO$	25			14.1
X	$CH_3SCH_2CH_2OH$	40		15	13.3
XI	$CH_3CH_2CH_2CH_2OH$	47		13	10.3

[a] ±20%. [b] $K_{m(aap)}$ of corresponding acetate.[4] [c] Calculated refraction volume of β substituent, $(CH_3)_3Si$, $(CH_3)_3C$, $(CH_3)_3N^+$, $(CH_3)_2S^+$, $(CH_3)_2N^+(O^-)$, $CH_3S(O_2)$, $CH_3S(O)$, CH_3S, or CH_3CH_2.[4] [d] Reference 7. [e] Bell, D., unpublished results. [f] This value has high uncertainty.

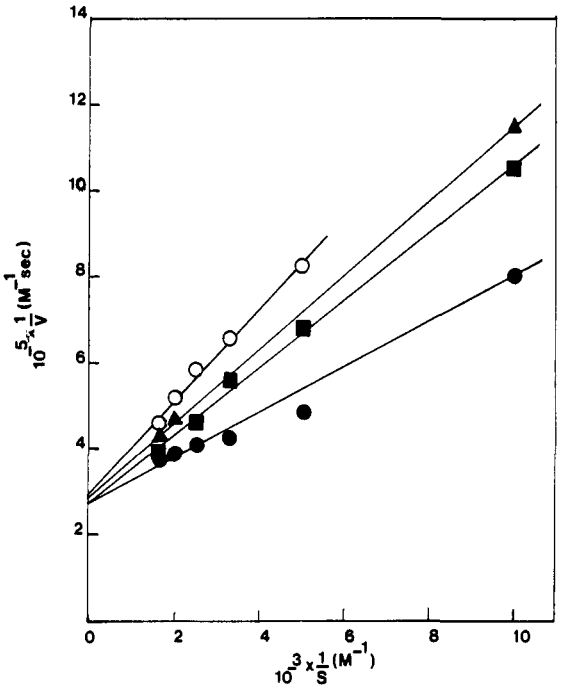

Figure 5. Inhibition by $CH_3S(O_2)CH_2CH_2OH$ (VII): ●, no inhibitor, ■, 4.5 mM VII; ▲, 6.75 mM VII; ○ 9 mM VII.

values of competitive ($K_{I(com)}$; these ratios of the secondary intercept vs. inhibitor concentration plots gave noncompetitive components, $K_{I(nonc)}$.[10] Values of binding constants for the inhibitors and related substrates and refraction volumes of β substituents are listed in Table I.

2-(Trimethylsilyl)ethanol (I) binds competitively, K_I = 3.3 mM, more strongly by 0.5 kcal/mol than its carbon analogue of smaller volume, 3,3-dimethylbutyl alcohol (IA), K_I = 7.5 mM, and the latter has a significant noncompetitive component. Binding of the silyl alcohol is the same as that of its acetate ester, and binding in the carbon alcohol and ester pair is also similar.

2-(Dimethylsulfonio)ethanol (II, "sulfocholine") binds largely competitively, with efficiency similar to that of its N analogue choline (IB), K_I = 0.4 mM; the latter has a somewhat larger noncompetitive component. Refraction volumes of the two cationic alcohols are similar, the larger S compensating for one less methyl. The acetate esters of IB and II, acetylcholine and acetylsulfocholine, have the same apparent binding constants, K_m, essentially the same as K_I values of the alcohols. However, high rates of acylation may displace the binding equilibria and true binding constants, K_s, may be slightly higher, ~1 mM for acetylcholine[5] and slightly less than this for acetylsulfocholine, with its lower value of k_{cat}.[4]

Trimethylsulfonium ion (III), K_I = 2.0 mM, in which a third methyl replaces the hydroxyethyl group of II, binds less well than II by 0.9 kcal/mol despite higher hydrophobicity and has a more significant noncompetitive component. Then, addition of an S–O bond, in conversion of III to trimethylsulfoxonium (IV), K_I = 1.3 mM, improved competitive binding slightly and removed the noncompetitive component.

On the other hand, introduction of the N^+–O^- bond greatly decreased binding. Trimethylamine oxide itself (V), examined up to 200 mM, showed no evidence of inhibition. When the $\equiv N^+O^-$ group was present in 1-(dimethylamino)-4-pentanone *N*-oxide (VI), a methyl ketone sterically similar to acetylcholine, competitive inhibition was observed, K_I = 11 mM, slightly more favorable than the corrsponding amine–oxide ester substrate, K_m = 18 mM,[4] but far weaker than the analogous ketone lacking the N^+–O^- group, 1-(dimethylammonio)-4-pentanone,[7] K_I = 0.77 mM; the amine–oxide group reduced binding by 1.5 kcal/mol.

Effect of the S–O bond on binding was further examined in 2-(methylsulfonyl)ethanol (VII). This binds moderately well, largely competitively, K_I = 8.7 mM, remarkably like the hydrocarbon analogue 2,3-dimethylbutyl alcohol (IA). As with the carbon analogues, the acetate of the sulfone alcohol, VIIA, showed binding similar to that of its alcohol, K_m = 6.2 mM when used as substrate,[4] K_I = 6.4 mM when examined as an inhibitor for hydrolysis of acetylcholine. The two S–O bonds in VII increase binding by a factor of 4.5, $\Delta\Delta G$ = 0.9 kcal/mol, over that of 2-(methylthio)-ethanol (X), K_i = 40 mM, while two C–CH_3 groups in IA increase binding over that of *n*-butyl alchol (XI), K_I = 47 mM, by a factor of 6.3, $\Delta\Delta G$ = 1.1 kcal/mol. Also the contribution of S in 2-(methylthio)ethanol to binding is essentially equivalent to that of CH_2 in *n*-butyl alcohol (XI).

Comparison of contributions of methyl and hydroxyethyl to binding of sulfones VII and VIII indicates hydroxyethyl to be superior by 0.7 kcal/mol. Comparison of compounds VIII and IX, dimethylsulfone and dimethyl sulfoxide, indicates that the second O of the sulfone makes no contribution in this weakly binding pair. However the 2-(methylsulfonyl)ethyl acetate substrate binds better than the sulfoxy substrate, K_m = 6.2 and 16 mM, respectively. The single S–O in Me_2SO (IX) appears to contribute to its binding, as its absence in 2-(methylthio)ethanol (X)

(10) Segal, I. H. "Enzyme Kinetics"; Wiley Interscience: New York, 1975; Chapter 3.

leads to slightly weaker binding, K_I = 25 and 40 mM, respectively, despite replacement of methyl by hydroxyethyl, which favors binding in comparisons of II with III and VII and VIII. However, the ester substrates derived from V and IX–XI have $K_m \sim 16$ mM, indicating essentially no distinctive effects on binding by these β substituents when present in acetate esters.

Discussion

That 2-(trimethylsilyl)ethanol and its acetate bind somewhat stronger than the carbon analogues, and values of k_{cat} for the esters are similar,[4] indicates some flexibility of the active site, allowing it to accommodate effectively both the volume of the silyl substituent and the different, possibly greater, distance between the β substituent and the acetoxyl. A common cause for noncompetitive inhibition, as by alcohols IA and IB, is binding to the acyl enzyme.[11-14] That inhibition by the silylalcohol has essentially no noncompetitive component may indicate that the longer C–Si bond does not allow it to fit into the acyl enzyme, the flexibility that accommodates the larger substrate not being sufficient for the separate, noncovalently attached, alcohol. Acetylsulfocholine and its alcohol, sulfocholine (II), with a trisubstituted β substituent, appear to bind as well as tetrasubstituted acetylcholine and its alcohol, choline (IB), but motion in the active site may be less well restricted, leading to slightly lower enzymic reactivity of the substrate.[4] The noncompetitive component of inhibition by the alcohol may arise from binding to the acyl enzyme.

In acetates of alcohols I, IA, IB, II, and VII, which have large β substituents that may fully occupy their subsite, the acetyl group makes no significant contribution to binding over that of the alcohols. However, the acetyl group in analogous isosteric ketones appears to lead to stronger binding.[7] Association of the enzyme serine hydroxyl oxygen with the ketone carbonyl carbon may occur directly, while that with the ester carbonyl may depend upon prior protonation of the alkoxy oxygen and loss of ester resonance.[5] On the other hand, in the acetates with small or weakly bound β substituents the ester group appears to dominate the binding, as the substrates with alkoxyl length corresponding in length to *n*-butyl, with β substituents $(CH_3)_2N^+(O^-)$, $CH_3N^+H_2$,[5] $CH_3S(O)$, CH_3S, and CH_3CH_2 all have similar binding, $K_m \sim 16$ mM. The hydroxyl and methylene of hyroxyethyl groups improve binding over methyl in the trialkylsulfonium compounds II and III, in the sulfones VII and VIII, and in the choline–tetramethylammonium pair,[5,11] despite lower hydrophobicity, π = −0.3 and +0.5, respectively.

The contrast between effect of N^+–O^- and –S(O)– bonds on binding is noteworthy. The first-row element N allows only the hydrated[16] dipolar structure, and ketone VI binds less strongly than small aliphatic ketones,[17] which may place no group in the trimethyl site. The amine–oxide bond leads to a 1.5 kcal/mol decrease in binding in the pair of ketones 1-(dimethylammonio)-4-pentanone and its *N*-oxide VI and in the pair of ester substrates 2-(dimethylammonio)ethyl acetate[5] and its *N*-oxide.[4] This effect of conversion of $\equiv NH^+$ to $\equiv N^+—O^-$ is similar to that observed in comparison of choline and sulfocholine with their uncharged analogues, I and IA.

Conversion of –S– to –S(O)– or $-S(O_2)-$ or $\equiv S^+$ to $\equiv S^+O$, on the other hand, increases binding. The bonding has dipolar and covalent character, $S^+—O^- \leftrightarrow S{=}O$,[18] with no net charge and no change in net charge from the thio or sulfonium state. The trisubstituted cations, trimethylsulfonium ion (III) and trimethylammonium ion, bind similarly, and both have substantial noncompetitive components.[11-13] Addition of O as the fourth substituent in $(CH_3)_3S^+O$ increases competitive inhibition and eliminates the noncompetitive component, much as the fourth methyl does in comparison of tetramethyl- and trimethylammonium ions.[11] Further, two oxygens of the sulfone alcohol (VII) and its ester increase binding over that of the thio alcohol (X) and its ester essentially as two methyl groups do in comparison of 3,3-dimethylbutanol and butanol (IA and X) and their esters. The sulfone oxygens have this effect although they greatly decrease hyrophobicity, Π, from +2.0 for $(CH_3)_3C$ to −1.9 for $CH_3S(O_2)$.[4,15] The sulfoxy and sulfone oxygens do not increase refraction volume, MR (Table I), with which property fit in the active site and reactivity have been correlated.[4] In this case MR may not be a relevant measure of volume but an artifact of the remarkably low absorption of the sulfone group.[18] van der Waals volumes,[19] however, indicate 34 cm^3 for $CH_3S(O_2)$, 24 for CH_3S, and 44 for $(CH_3)_3C$. Thus, volume and fit in the subsite may contribute importantly to effective sulfone binding. The ability of sulfones to permeate and associate with both aqueous and lipid phases may lead to cholinesterase modifying and other biologically active compounds.

It may be noted that (2-hydroxyethyl)dimethylsulfoxonium ion, the hydroxyethyl analogue of compound IV, has physiologic action as the allergenic agent causing Dogger Bank itch.[20] We would expect this compound to bind to AcChE more strongly than compound IV, with an estimated K_I of ~0.2 mM. However, incubation of AcChE with compound IV led to no irreversible inhibition, indicating that the trimethyl binding site contains no reactive nucleophile that may be methylated[21] by IV.

Binding to AcChE is favored by cationic charge and spherical substituent shape of a certain size range. It is not dependent on hyrophobic surface character but, apparently, on less specific dispersion forces. Thus, it appears that while the receptor responds rather specifically to acetylcholine and closely related structures, the enzyme stands nearby ready to bind and try to hydrolyze and remove compounds that even superficially resemble the natural agonist and might cause erroneous receptor response.

Materials and Methods

2-(Trimethylsilyl)ethanol, $(CH_3)_3SiCH_2CH_2OH$ (I, Fluka, purum), was redistilled. 3,3-Dimethylbutanol and choline (IA and IB) were available from previous work.[7] 2-(Dimethylsulfonio)ethanol iodide $(CH_3)_2{}^+SCH_2CH_2OHI^-$ (II) was prepared from 0.072

(11) Krupka, R. M. *Biochemistry* **1965**, *4*, 429–435.
(12) Krupka, R. M.; Hellenbrand, K. *Biochim. Biophys. Acta* **1974**, *370*, 208–215.
(13) Wilson, I. B.; Alexander, J. *J. Biol. Chem.* **1962**, *237*, 1323–1326.
(14) Dawson, R. M. *J. Neurochem.* **1978**, *30*, 865–870.
(15) Hansch, C.; Leo, A. "Substituent Constants for Correlation Analysis in Chemistry and Biology"; Wiley: New York, 1979.
(16) Stewart, T. D.; Maeser, S. *J. Am. Chem. Soc.* **1924**, *46*, 2585–2590.
(17) Dafforn, A.; Jewell, M.; Anderson, M.; Ash, D.; Horvath, D.; Kitson, R.; Margiotta, S.; Ryck, G. *Biochim. Biophys. Acta* **1979**, *569*, 23–30.
(18) Price, C. C.; Oae, S. "Sulfur Bonding"; Ronald Press: New York, 1962; Chapters 3 and 4.
(19) Bondi, A. "Physical Properties of Molecular Crystals, Liquids and Gasses"; Wiley: New York, 1968; Chapter 14.
(20) Carle, J. S.; Christophersen, C. *J. Am. Chem. Soc.* **1980**, *102*, 5108–5109.
(21) Kuhn, R.; Trischmann, H. *Ann.* **1958**, *611*, 119–121.

mol of 2-(methylthio)ethanol (Aldrich) and 0.13 mol of methyl iodide (Fisher), at 25 °C for 48 h. The mixture was treated with 10 mL of acetone, cooled to 0 °C, and filtered, and the product was crystallized from ethanol–acetone, mp 58 °C, lit.[22] mp 55 °C. Trimethylsulfonium iodide, $(CH_3)_3S^+I^-$ (III), trimethylsulfoxonium iodide $(CH_3)_3S^+OI^-$ (IV), and dimethylsulfone (VIII) were obtained from Aldrich and recrystallized from water, mp 211, 170, and 109 °C, respectively. (2-Hydroxyethyl)methylsulfone, $CH_3S(O_2)CH_2CH_2OH$ (VII, Fluka, Purum), was used directly. Its acetate, VIIA, was available from previous work.[4] Dimethyl sulfoxide (IX), 2-(methylthio)ethanol (X), and butanol (XI) were obtained from Aldrich and redistilled. Trimethylamine oxide hydrochloride, $(CH_3)_3N^+O^-HCl$ (V) (Aldrich), was recrystallized from ethanol, mp 214 °C. 1-(Dimethylamino)-4-pentanone *N*-oxide, $(CH_3)_2N^+(O^-)CH_2CH_2CH_2COCH_3 \cdot HCl$ (VI), was prepared by treatment of 3.0 g (0.023 mol) of 1-(dimethylamino)-4-pentanone (Sapon) with 5 g (0.03 mol) of *m*-chloroperbenzoic acid in 65 mL of dry benzene at 25 °C for 5 days. Dry ether, saturated with HCl (20 mL), was added, the mixture was cooled, and the precipitated oil and solid were washed with ether and crystallized from 1-butanol–hexane and 1-butanol–ether: mp 105–106 °C; 2.3 g (54% yield).

Anal. Calcd for $C_7H_{16}NO_2Cl$: C, 46.3; H, 8.82; N, 7.71. Found: C, 46.2; H, 8.79; N, 7.83 (Galbraith).

Kinetic studies were carried out as described previously[5] at pH 7.8, 25 °C, in 0.18 M NaCl, under nitrogen or argon. Acetylcholinesterase (EC 3.1.1.7, V-S, lyophilized, Sigma), ~1300 units, was dissolved in 10 mL of 0.18 M NaCl and stored at 4 °C. An aliquot was assayed before each inhibitor study by hydrolysis of acetylcholine iodide (Aldrich) recrystallized (mp 160–161 °C) with $k_{cat} = 1.6 \times 10^4\ s^{-1}$. Rates of hydrolysis of three to six concentrations (~0.1–0.6 mM) of acetylcholine by 10^{-9}–10^{-10} M enzyme were obtained at three to four concentrations of inhibitor.

Least-squares analysis of inverse rate vs. inverse substrate concentration led to slope and intercept at each concentration of inhibitor. Average errors in slopes were ±2–5%, in intercepts ±4–20%, correlation 0.98–0.99. Sets of three figures follow: inhibitor concentration, slope and intercept of $1/V$ vs. $1/S$ plots. Inhibitor I, $(CH_3)_3SiCH_2CH_2OH$: 0 mM, 42 s, $4.9 \times 10^5\ M^{-1}$ s; 1.81 mM, 54 s, $5.2 \times 10^5\ M^{-1}$ s; 4.52 mM, 80 s, $5.0 \times 10^5\ M^{-1}$ s; 11.9 mM, 160 s, $5.3 \times 10^5\ M^{-1}$ s; 17.9 mM, 230 s, $5.1 \times 10^5\ M^{-1}$ s. Inhibitor II, $(CH_3)_2S^+CH_2CH_2OHI^-$: 0 mM, 110 s, $4.9 \times 10^5\ M^{-1}$ s; 2.25 mM, 540 s, $6.0 \times 10^5\ M^{-1}$ s; 4.5 mM, 900 s, $7.6 \times 10^5\ M^{-1}$ s; 6.75 mM, 1500 s, $7.3 \times 10^5\ M^{-1}$ s. Inhibitor III, $(CH_3)_3S^+I^-$: 0 mM, 44 s, $2.5 \times 10^5\ M^{-1}$ s; 2.47 mM, 120 s, $3.7 \times 10^5\ M^{-1}$ s; 4.93 mM, 185 s, $4.7 \times 10^5\ M^{-1}$ s; 7.39 mM, 230 s, $5.2 \times 10^5\ M^{-1}$ s. Inhibitor IV, $(CH_3)_3S^+OI^-$: 0 mM, 108 s, $3.9 \times 10^5\ M^{-1}$ s; 2.25 mM, 277 s, $4.0 \times 10^5\ M^{-1}$ s; 4.5 mM, 435 s, $3.8 \times 10^5\ M^{-1}$ s; 6.75 mM, 640 s, $4.2 \times 10^5\ M^{-1}$ s. Inhibitor VI, $(CH_3)_2N^+(O^-)CH_2CH_2CH_2COCH_3$: 0 mM, 94 s, $2.5 \times 10^5\ M^{-1}$ s; 4.5 mM, 156 s, $1.9 \times 10^5\ M^{-1}$ s; 9.0 mM, 180 s, $2.1 \times 10^5\ M^{-1}$ s; 18 mM, 240 s, $2.7 \times 10^5\ M^{-1}$ s; 27 mM, 320 s, $1.7 \times 10^5\ M^{-1}$ s. Inhibitor VII, $CH_3S(O_2)CH_2CH_2OH$: 0 mM, 51 s, $2.8 \times 10^5\ M^{-1}$ s; 4.5 mM, 79 s, $2.7 \times 10^5\ M^{-1}$ s; 6.75 mM, 86 s, $2.9 \times 10^5\ M^{-1}$ s; 9.0 mM, 106 s, $3.0 \times 10^5\ M^{-1}$ s. Inhibitor VIIA, $CH_3S(O_2)CH_2CH_2OCOCH_3$: 0 mM, 60 s, $3.2 \times 10^5\ M^{-1}$ s; 2.25 mM, 94 s, $3.6 \times 10^5\ M^{-1}$ s; 4.5 mM, 110 s, $4.4 \times 10^5\ M^{-1}$ s; 6.75 mM, 130 s, $5.0 \times 10^5\ M^{-1}$ s. Inhibitor VIII, $(CH_3)_2SO_2$: 0 mM, 45 s, $1.9 \times 10^5\ M^{-1}$ s; 49.5 mM, 110 s, $2.3 \times 10^5\ M^{-1}$ s, 99 mM, 180 s, $2.5 \times 10^5\ M^{-1}$ s; 161 mM, 280 s, $3.1 \times 10^5\ M^{-1}$ s. Inhibitor IX, $(CH_3)_2SO$: 0 mM, 46 s, $2.1 \times 10^5\ M^{-1}$ s; 15.8 mM, 62 s, $2.2 \times 10^5\ M^{-1}$ s; 36.8 mM, 99 s, $1.9 \times 10^5\ M^{-1}$ s; 52.5 mM, 132 s, $1.9 \times 10^5\ M^{-1}$ s. Inhibitor X, $CH_3SCH_2CH_2OH$: 0 mM, 57 s, $3.1 \times 10^5\ M^{-1}$ s; 47 mM, 100 s, $3.1 \times 10^5\ M^{-1}$ s; 94 mM, 175 s, $2.8 \times 10^5\ M^{-1}$ s; 141 mM, 233 s, $3.0 \times 10^5\ M^{-1}$ s. Inhibitor XI, $CH_3CH_2CH_2CH_2OH$: 0 mM, 97 s, $3.8 \times 10^5\ M^{-1}$ s; 36.5 mM, 195 s, $2.5 \times 10^5\ M^{-1}$ s; 73 mM, 200 s, $3.7 \times 10^5\ M^{-1}$ s; 110 mM, 310 s, $3.7 \times 10^5\ M^{-1}$ s; 146 mM 410 s, $2.9 \times 10^5\ M^{-1}$ s.

Slopes and intercepts of plots of secondary slopes of the $1/V$ vs. $1/S$ data against inhibitor concentrations were calculated; errors in these slopes were ±2–10%, in the intercepts, ±3–13%, correlation 0.98–0.99. Values of slope and intercept follow for each inhibitor: I, $1.08 \times 10^4\ M^{-1}$ s, 36 s; II, $2.00 \times 10^5\ M^{-1}$ s, 83 s; III, $2.53 \times 10^4\ M^{-1}$ s, 51 s; IV, $7.8 \times 10^4\ M^{-1}$, 102 s; VI, $7.8 \times 10^3\ M^{-1}$ s, 106 s; VII, $5.9 \times 10^3\ M^{-1}$ s, 51 s; VIIA, $10.0 \times 10^3\ M^{-1}$ s, 65 s; VIII, $1.46 \times 10^3\ M^{-1}$ s, 41 s; IX, $1.66 \times 10^3\ M^{-1}$ s, 41 s; X, $1.28 \times 10^3\ M^{-1}$ s, 51 s; XI, $2.01 \times 10^3\ M^{-1}$ s, 95 s. Slopes and intercepts of plots of intercept against inhibitor concentrations were calculated for inhibitors II, III, VII, VIIA, and VIII; errors in these secondary slopes were ±2–7%, in the intercepts, ±2–9%, correlation 0.98–99. Values of these slopes and intercepts follow: II, $3.9 \times 10^7\ M^{-2}$ s, $5.1 \times 10^5\ M^{-1}$ s; III, $3.7 \times 10^7\ M^{-2}$ s, $2.66 \times 10^5\ M^{-1}$ s; VII, $2.3 \times 10^6\ M^{-2}$ s, $2.7 \times 10^5\ M^{-1}$ s; VIIA, $2.8 \times 10^7\ M^{-2}$ s, $3.12 \times 10^5\ M^{-1}$ s; VIII, $7.2 \times 10^5\ M^{-2}$ s, $1.9 \times 10^5\ M^{-1}$ s.

Refraction volumes, MR, were calculated as described in ref 4 from tabulated refractive index and atomic and group MR additive values.[23,15]

Hydrophobicity, Π.[15] Values of Π in aliphatic compounds are CH_3, +0.50; CH_2CH_3, +1.0; and HO, -1.3. Values for aliphatic $CH_3S(O_2)$ and $(CH_3)_3C$ are discussed in ref 4.

van der Waals volumes, calculated from bond distances and van der Waals radii, were derived from tabulated values:[19] CH_3, 13.7 cm^3; -S- 10.8 cm^3; $-S(O_2)-$, 20.3 cm^3.

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Registry No. I, 2916-68-9; IA, 624-95-3; IB, 67-48-1; II, 25059-71-6; III, 2181-42-2; IV, 1774-47-6; V, 7651-88-9; VI, 97315-41-8; VIi, 15205-66-0; VIIA, 92543-10-7; VIII, 67-71-0; IX, 67-68-5; X, 5271-38-5; XI, 71-36-3; acetylcholinesterase, 9000-81-1; 1-(dimethylamino)-4-pentanone, 43018-61-7; *m*-chloroperbenzoic acid, 937-14-4.

(22) Jilek, J. O.; Protiva, M. *Czechoslav. Farm.* **1957**, *6*, 113–119, *Chem. Abst.* **1957**, *51*, 14596*h*.

(23) Vogel, A. I. *J. Chem. Soc.* **1948**, 1820–1854 and preceding papers.