Inhibition of Acetylcholinesterase by Diastereomers of 2-Methylamino-1-phenylpropanol and Their Derivatives

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The anti-acetylcholinesterase activities of the ephedrine diastereomers and their *N*-methyl derivatives were correlated to the conformation of the molecules in solution. The stereospecificity exhibited by enantiomers of *N*-methyl-ψ-ephedrine was attributed to the predominance of one preferred conformation. The possibility of predicting the absolute configuration of chiral inhibitors from enzyme inhibitions data is discussed.

Keywords anti-acetylcholinesterase; ephedrine; ψ-ephedrine; diastereomer; stereospecificity; conformation; absolute configuration

Aminoethanols are generally good inhibitors of acetylcholinesterase (AChE) because their amino function is protonated at physiological pH, and this positively charged nitrogen is separated from the inherently negative hydroxyl oxygen by two carbon atoms, similar to the N^+–C–C–O segment of acetylcholine (ACh). The positively charged nitrogen interacts with the anionic site of the enzyme, while the electro-negative oxygen interacts with an acid group *via* hydrogen bonding.

In our recent review of the structure of the AChE active center and the mode of ACh/AChE interaction,[1] we proposed that the acid group of the receptor is located 3—4 Å away from the centre of the anionic site in a three-dimensional structure to which the ACh molecule, with τ N–C–C–O = +60° to +137°, can bind. Thus for good fitting onto the active center of AChE, the N–C–C–O segment of a substrate or inhibitor must have the appropriate absolute conformation. This is perhaps the critical factor that distinguishes a good substrate or inhibitor from its optical antipode, and this has been observed in a number of chiral aminoethanols. Thus, the more potent anti-AChE activity of (−)-mefloquine as compared to its optical antipode has been attributed to the positive synclinal conformation of the N–C–C–O segment of the former.[2] Similarly (+)-quinidine was also found to be more active than (−)-quinine. These two compounds are configurational analogues of (−)-mefloquine and (+)-mefloquine respectively.[2]

As a follow-up to the above studies, we studied another series of aminoethanols—the ephedrines and ψ-ephedrines, which are the diastereomers of 2-methylamino-1-phenylpropanol. In addition, the tertiary amino and quaternary ammonium derivatives of these compounds were also evaluated for inhibitory activities toward AChE. A correlation study of the anti-AChE activities of these compounds with the absolute conformation of their N–C–C–O segment would be interesting.

Experimental

Materials The enantiomers of ephedrine, ψ-ephedrine and their *N*-methyl derivatives were purchased from Sigma. The specific rotations of these compounds were confirmed by polarimetric measurements. (+)-*N*-Methylephedrine, which was not available commercially, was synthesized by the reported method.[3] Quaternization of the four tertiary amines was achieved in ether with excess methyl iodide. The quaternary products on recrystallization from ethanol gave melting point values which were identical to literature values.[4]

Enzyme Inhibition Studies The anti-AChE activities of these compounds at pH 7.4 were determined by the pH-stat method as described previously.[2] The results were analyzed by means of ROSFIT, a computer program written for the discrimination between rival inhibitory models and for estimation of kinetic parameters.[5] The inhibitory constants of the compounds are listed in Table I.

Nuclear Magnetic Resonance (NMR) Studies The NMR spectra of the protonated species of (+)-*N*-methylephedrine and (+)-*N*-methyl-ψ-

TABLE I. Inhibition of AChE by Ephedrine Stereoisomers

Compounds	K_i ($\times 10^{-4}$ M)[a]	Stereospecificity ratio[b]	Mode of inhibition
(−)Ephedrine	14.65 (1.47)	1.47	Competitive
(+)Ephedrine	9.94 (1.01)		
(−)ψ-Ephedrine	24.78 (2.39)	1.23	Competitive
(+)ψ-Ephedrine	20.13 (2.24)		
(−)*N*-Methylephedrine	8.85 (0.87)	1.46	Competitive
(+)*N*-Methylephedrine	12.95 (1.06)		
(−)*N*-Methyl-ψ-ephedrine	6.28 (0.35)	3.86	Non-competitive
(+)*N*-Methyl-ψ-ephedrine	24.24 (0.86)		
(−)*N*-Methyl-ephedrine methiodide	13.45 (0.68)	1.48	Competitive
(+)*N*-Methyl-ephedrine methiodide	19.87 (1.85)		
(−)*N*-Methyl-ψ-ephedrine methiodide	7.99 (0.61)	1.72	Competitive
(+)*N*-Methyl-ψ-ephedrine methiodide	4.64 (0.23)		

a) Values in parentheses indicate S.E.M. for 20 observations obtained using at least 2 different concentrations of inhibitor. *b*) Ratio of the K_i values of a pair of enantiomers with the lower K_i value as denominator. A ratio of more than 2 indicates pronounced stereospecificity.

TABLE II. Vicinal Coupling Constants of Protonated Ephedrine Diastereomers and Their *N*-Alkyl Derivatives

	Solvent	J_{ab}(Hz)[b]	Calculated conformer population (%)[c]
Ephedrine	D_2O	3.63	E1, E2 (90); E3 (10)
N-Methylephedrine[a]	D_2O	3.42	E1, E2 (92); E3 (8)
ψ-Ephedrine	D_2O	9.21	T1 (83—85); T2, T3 (17—15)
N-Methyl-ψ-ephedrine[a]	D_2O	10.25	T1 (97); T2, T3 (3)

a) The tertiary base was protonated with trifluoroacetic acid (TFA) before dissolving it in D_2O. *b*) Coupling constant measured from the doublet of the C-1 proton. *c*) Calculated according to Portoghese.[13] Values for ephedrine and ψ-ephedrine have been reported by Portoghese.[13]

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ephedrine were obtained with a JEOL FX 90Q-FT NMR spectrometer at an operating frequency of 90 MHz. The vicinal coupling constants between the protons at C1 and C2 are given in Table II.

Results and Discussion

Of the six enantiomeric pairs of ephedrine stereoisomers evaluated, only the *N*-methyl-ψ-ephedrines showed pronounced stereospecificity in their anti-AChE activities (Table I): (−)-*N*-methyl-ψ-ephedrine was found to be 3.86 times more active than its optical antipode.

In general, the key prerequisites for stereospecificity in drug/receptor interaction are (a) there is a preferred and predominant conformation of the drug molecules in solution and (b) the absolute conformation of one enantiomer fits the receptor well while that of its optical antipode does not. A discussion of the enzyme inhibition data from this study would be meaningless unless these two factors are taken into consideration.

Preferred Conformation of Ephedrine and ψ-Ephedrine Conformational studies on ephedrine and ψ-ephedrine have been well reported in the literature.[6−13] Chart 1 illustrates the three possible staggered conformations about the central C–C bond of ephedrine and ψ-ephedrine. An X-ray analysis has shown that the preferred conformation of ephedrine salts is E1,[6,7] and that of ψ-ephedrine and its hydrochoride salt is T1.[8] Quantum mechanical calculation on the ephedrine molecule indicates that E1 and E2 are of comparable stabilities, with a higher probability for E1.[9] Kier, on the basis of an extended Huckel calculation, suggested that the preferred conformation of ψ-ephedrine was T2,[10] which is doubtful in view of the contrary X-ray data which favor T1.[8]

In terms of drug/receptor interaction, what is more important is the preferred conformation of the molecules in solution. Based on infrared studies in solutions, Kanzawa proposed that the hydroxyl and methylamino functions of both ephedrine and ψ-ephedrine were in gauche conformations in chloroform and carbon tetrachloride due to intramolecular hydrogen bondings of the type OH···N.[11] It was also noted that ψ-ephedrine formed stronger intramolecular hydrogen bonds. Early NMR studies by Hyne gave the following conformational distributions in solution of the ephedrine diastereomers: ephedrine—E1 (40%), E2 (40%), E3 (20%); and ψ-ephedrine—T1 (62%), T2 (30%), T3 (8%).[12] Subsequent NMR studies by Portoghese showed that in a variety of solvents, the ephedrine diastereomers were intramolecularly hydrogen bonded both as free bases and protonated species in very much the same conformation.[13] He calculated that ephedrine hydrochloride in D_2O contained approximately 90% E1 and E2, and 10% E3, while ψ-ephedrine hydrochloride possessed 83—85% T1 and 17—15% T2 and T3. As these aliphatic bases are protonated at physiological pH, the above estimate of the conformer ratio of the protonated species in solution is particularly useful to the present study.

Further evidence from NMR studies indicated that *N*-alkylation with bulky groups did not affect the conformations of the ephedrine diastereomers.[14] This was attributed to strong intramolecular hydrogen bonding. As these studies only involved free bases, it was necessary to find out whether the corresponding protonated species behave similarly. Thus, the NMR spectra of the protonated species of *N*-methylephedrine and *N*-methyl-ψ-ephedrine were studied. The relative population ratio of the three staggered rotamers were computed from the observed vicinal coupling constants between the C1 and C2 protons according to Portoghese.[13] It can been seen (Table II) that the preferred conformation of the protonated *N*-methyl-ψ-ephedrine in D_2O is T1 (97%), with a greater preference than that of the protonated ψ-ephedrine (83%).[13] This preferred conformation is probably due to (a) strong intramolecular hydrogen bonding of the type $N^+H\cdots O$ which favors T1 and T2 against T3, and (b) the presence

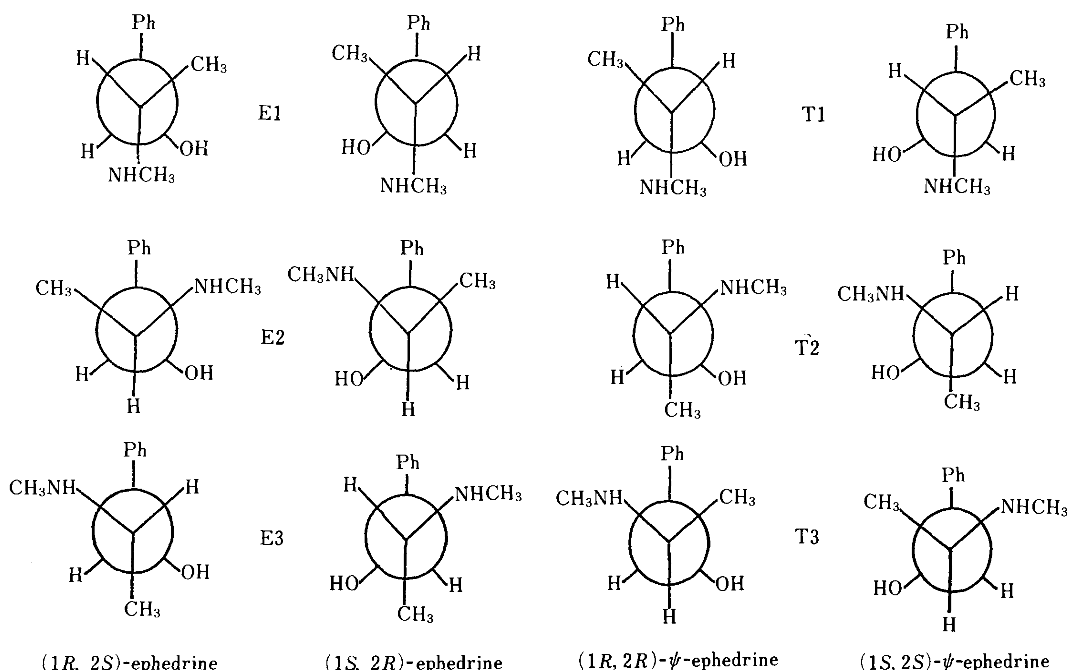

Chart 1

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of the fewest nonbonded interactions which also favors T1 against T2 and T3. As *N*-methyl-ψ-ephedrine has a bulkier dimethylamino group, it is not surprising that its population of T1 rotamer is greater than that of ψ-ephedrine.

With regard to protonated ephedrine and *N*-methylephedrine, calculation from NMR data showed a predominance of the E1 and E2 conformers (90—92%). Unfortunately, it was not possible to compute the relative proportion of E1 and E2. In this respect, one may perhaps rely on a reported quantum mechanical calculation of the ephedrine molecule which indicates that E1 and E2 are of comparable stabilities, with a higher probability for E1.[9]

As the protonated ephedrine and *N*-methylephedrine are unlikely to have a predominant conformation, one would not expect them to be stereospecific in their anti-AChE activity. This has been found to be the case in the present study (Table I). ψ-Ephedrine and *N*-methyl-ψ-ephedrine, on the other hand, have a predominant T1 conformation, for which stereospecificity is expected. This was found to be true with *N*-methyl-ψ-ephedrine, but not ψ-ephedrine or the quaternary derivatives

As the ψ-ephedrine enantiomers only have one *N*-methyl group to interact with the anionic site of the enzyme active center, their weak inhibitory activities were expected. For good interaction with the anionic site, two *N*-methyl groups are necessary.[1] Unfortunately, weak inhibitory activities tend to obscure stereospecificity, if any, and this has been found to be the case.

With the quaternary ψ-ephedrinium enantiomers, the absence of strong intramolecular $N^+H \cdots OH$ hydrogen bonding has removed an important factor determining the preferred conformation of ψ-ephedrine. The predominance of the T1 rotamer is less likely and this may explain the lack of stereospecificity. It is interesting to note that the K_i values of the quaternary compounds are close to that of the analogous choline ($K_i = 4.5 \times 10^{-4}$),[15] which is achiral.

It is remarkable to note that the enantiomeric pair of *N*-methyl-ψ-ephedrine, which alone exhibits pronounced stereospecificity in enzyme–inhibitory activity, is also alone in showing a noncompetitive mode of inhibition. In this respect one should mention that mefloquine, quinine, quinidine, which were reported to exhibit stereospecificity in their inhibition of AChE,[2] are also noncompetitive inhibitors. Indeed, certain achiral compounds such as amodiaquine and its derivatives have also been reported to be noncompetitive inhibitors of AChE.[16] Despite their diversity of structures, these non-competitive inhibitors share two common characteristics: (a) the possession of a protonated tertiary ammonium group at physiological pH and (b) the existence of strong intramolecular hydrogen bonding of the type $N^+H \cdots OH$. We have reviewed this phenomenom previously and speculated that noncompetitive inhibitors of this type interact with the same anionic site as the competitive inhibitors.[1] Their presence at the anionic site inhibits the deacetylation step of the acetylated enzyme, thus giving rise to the noncompetitive mode of inhibition. It is unclear how these compounds inhibit the deacetylation step. Perhaps the answer may lie in their strong intramolecular hydrogen bonding which introduces an element of conformational rigidity to the enzyme–inhibitor complex. Most postulates of the mechanism of enzymatic hydrolysis of ACh have suggested conformational changes on the part of the enzyme during hydrolysis.[17]

The Absolute Configuration of (−)-*N*-Methyl-ψ-ephedrine The stereospecific anti-AChE activities of the *N*-methyl-ψ-ephedrine enantiomers are also interesting with respect to their stereochemistry. When the absolute configuration of an enantiomeric pair is known, one can by knowing their preferred conformations, deduced their absolute conformations. Furthermore, one can, by knowing which enantiomer is a better enzyme inhibitor, predict the absolute conformational requirement of the enzyme active center. This is provided that the inhibitor interacts with the enzyme active center in its preferred conformation. Conversely, when the absolute conformational requirement of the active site is known with some confidence, one can, by knowing which member of an enantiomeric pair is more active, predict its absolute configuration. (−)-*N*-Methyl-ψ-ephedrine is an interesting case in point. It is the more active enantiomer, and its preferred conformation in D_2O is T1. The enantiomeric pair of *N*-methyl-ψ-ephedrine have 1*R*,2*R* or 1*S*,2*S* as their absolute configuration. The torsion angle of the N–C–C–O segment of the 1*S*,2*S* enantiomer is $+60°$ with respect to conformation T1, and that of its optical antipode (1*R*,2*R*) $-60°$. As our previous studies have indicated that positive N–C–C–O torsion angle is essential for good fit onto the active site of AChE,[1,2] one can predict that (−)-*N*-methyl-ψ-ephedrine, being the more active enantiomer, should have the absolute configuration of 1*S*,2*S* and its optical antipode 1*R*,2*R*. This prediction unfortunately contradicts the currently accepted assignment based on chemical studies.[18,19]

There can be only two explanations. First, the above tentative approach to absolute configuration determination is unreliable as it is based on the unproven assumption that the inhibitor interacts with the enzyme active center in its preferred conformation. The possibility of induced fit has not been discounted. Second, the hitherto accepted assignment of the absolute configuration of the ψ-ephedrine enantiomers may not be correct.

The latter explanation is indeed heretical at first sight. However, a survey of the literature reveals that the validity of the original configurational assignment by Freudenberg and Nikolai[18] and Leithe[19] has been disputed over a period of time by other investigators.[20-22] Though Ramachandran and Raman confirmed the absolute configuration of (+)-ephedrine by X-ray analysis,[23] and there have been concurring studies with circular dichroism (CD),[24,25] similar investigations on ψ-ephedrine are few. In this respect, one should mention that Mathew and Palenik had made an unsuccessful attempt to determine the absolute configuration of (+)-ψ-ephedrine by X-ray analysis.[8] Their results were said to be inconclusive, which is surprising.

It would appear that the present contradictory assignment based on enzyme kinetic studies does cast some nagging doubt on past assignment of the absolute configuration of ψ-ephedrine. The resolution of this contradiction requires further X-ray studies based on the current state of the art. Nevertheless, the suggested use of enzyme kinetic data to predict the absolute configuration of chiral inhibitors (or substrates) of enzyme is worthy of

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further investigation.

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