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Molecular determinants in the bioactivation of the dopaminergic neurotoxin *N*-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)

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SUMMARY

Nineteen analogs of the dopaminergic neurotoxin *N*-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) have been used as probes to study the structural parameters that influence MAO-catalyzed oxidation. In this study, the efficiency of enzyme-catalyzed substrate oxidation was found to be unrelated to parameters such as the ionization potential, dipole moment, net atomic charge at C5 and the dihedral angle between the phenyl ring and the tetrahydropyridine moiety. Conformational analysis revealed that substitution at the C2′ position of MPTP yields atropisomers. It is suggested that one of these atropisomers would be either inactive or substantially less active than the other. Therefore, the relative oxidative efficiency and toxicity of these compounds reported earlier may have been significantly underestimated. Based on the conformational analysis and other data, a rudimentary model of the MAO substrate site has been developed which partially explains the substrate specificities of MAO A and MAO B.

Each substrate binding site can be divided into two regions, (a) an amine-binding pocket (for the tetrahydropyridine moiety), and (b) a 'bulky substituent' region (for the phenyl group and its substituents). The length of the substrate binding site (measured along the long axis of MPTP) is approximately 8.5 Å, and the width of the 'amine-binding' pocket is approximately 2.5 Å (from C3 to C5). The 'bulky substituent' region contains a central area for binding the phenyl group of MPTP. This central area is flanked by two hydrophobic pockets, P2′ and P3′. In MAO A, the pocket P2′-A is oriented 45–135° relative to the plane of the tetrahydropyridine moiety, with a radius of 3.1 Å from C2′ of the phenyl ring. The radius of a similar but smaller pocket, P2′-B, in MAO B, is approximately 2.7 Å. In MAO B, the pocket P3′-B (radius 2.36 Å from C3′) is larger than a similar pocket P3′-A (radius 1.70 Å from C3′) in MAO A. The foregoing characterization suggests that differences in the size and topography of both of the substituent pockets play an important role in determining the substrate specificities of these two isozymes.

INTRODUCTION

N-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) induces a Parkinsonian syndrome in humans and nonhuman primates [1,2,3]. The MAO-catalyzed bioactivation of *N*-methyl-4-

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phenyl-1,2,3,6-tetrahydropyridine (MPTP) yields the cationic metabolite and putative dopaminergic neurotoxin *N*-methyl-4-phenylpyridinium (MPP^+) [4,5]. The selective dopaminergic neurotoxicity of MPP^+ is thus partly explained by its selective accumulation within catecholaminergic neurons via the dopamine re-uptake system [6,7,8]. A direct consequence of this selectivity is that central dopaminergic neurons maintain higher concentrations of MPP^+ relative to the rest of the brain [9,10]. At these high concentrations, MPP^+ successfully inhibits a number of key metabolic processes within the cell, resulting in its destruction (for reviews, see Refs. [11–14]).

Several analogs of MPTP have been synthesized and evaluated in MAO assays and in vivo [15–25]. Among these may be found both poor and excellent substrates of MAO. Poor substrates of MAO invariably fail to exhibit dopaminergic neurotoxicity, presumably due to absence of bioactivation. However, at least one excellent substrate of MAO, 4-homo-MPTP, was also found to be nontoxic [21]. From these studies, it has been concluded that MAO-catalyzed bioactivation is necessary, but not sufficient for neurotoxicity. However, the stereo-electronic parameters underlying the bioactivation of these analogs remain largely unknown. A recent structure-activity study [26] has provided some insight in this area. Based on the view that MAO-catalyzed bioactivation is a pivotal step in MPTP-induced neurotoxicity, we have initiated studies to better describe those properties which are important for the bioactivation of an MPTP analog. Specifically, we have used a combination of recently reported structure-activity data [26], semi-empirical quantum mechanical calculations and conformational studies, to derive a model of the substrate binding sites of MAO A and B. Models such as this may help explain the relative importance of these isozymes in the bioactivation/detoxification of potential neurotoxins and other xenobiotics.

METHODS

With the assistance of Cray Research Laboratories (Mendota Heights, MN) and the Minnesota Supercomputer Center, the semi-empirical quantum mechanical program MOPAC4 (general molecular orbital package) was vectorized and ported to a 4-processor 256-megaword Cray 2 supercomputer with a theoretical performance of 1 gigaFLOP. In order to verify correct operation of the program, the test files supplied were used as input data and the results scrutinized. In addition, we compared the results from two analogs of MPTP with those previously obtained using a Cray XMP (Cray Research Laboratories). The RHF/AM1 method [36] and Cartesian coordinates were used for all calculations, unless otherwise specified. The criterion for completion for all calculations was a scalar gradient of 0.1 (GNORM = 0.1). The results were post-processed on a Silicon Graphics 7D/GT workstation using the program MIDAS (UCSF). To obtain rotational barriers, minimizations were done for selected values of the ring-ring dihedral angle (from 0° to 180° in steps of 10° or 20°). The heats of formation were then plotted against the value of the dihedral angle. The dihedral angle used in this study is defined by C3-C4-C1′-C2′. For the analog 4-homo-MPTP, a comparable angle is C3-C4-C8-C1′ (see Fig. 1). Molecular comparison was

Abbreviations: MAO, monoamine oxidase; MPTP, *N*-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; 2′-MeMPTP, *N*-methyl-4-(2-methylphenyl)-1,2,3,6-tetrahydropyridine or 2′-methyl-MPTP; MNTP, *N*-methyl-4-(1-naphthyl)methyl-1,2,3,6-tetrahydropyridine; MPHTP, *N*-methyl-4-(9-phenanthryl)methyl-1,2,3,6-tetrahydropyridine; MCTP, *N*-methyl-4-cyclohexyl-1,2,3,6-tetrahydropyridine; EPTP, *N*-ethyl-4-phenyl-1,2,3,6-tetrahydropyridine; PPTP, *N*-propyl-4-phenyl-1,2,3,6-tetrahydropyridine.

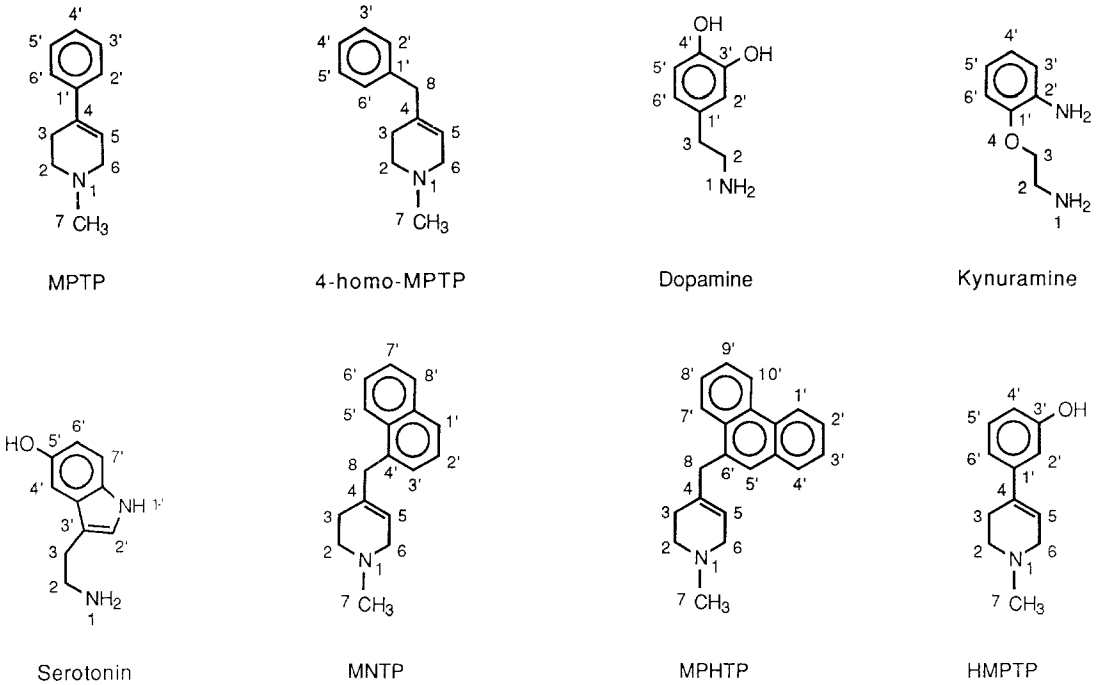

Fig. 1. Structures of MPTP, MPTP analogs and selected arylalkylamines.

effected by least-squares fitting with the software package PCMODEL. Graphic display was effected through PCMODEL (Serena Software, Bloomington, IN) using an Intel 386/387-based microcomputer.

RESULTS

Structural aspects of MPTP

Pertinent data from the semiempirical MO calculations, combined with the biological data recently reported [26], are summarized in Table 1. The relative activity is derived from the parameter Turnover number/k_m normalized to MPTP. In the minimum energy structure of MPTP (Fig. 2A,B), the tetrahydropyridine appears as an envelope with a twisted flap. The atoms N1 and C2 are located on opposite sides of a plane which is defined by carbon atoms C3, C4, C5 and C6. The ring-ring dihedral angle is 49.2°. Earlier semi-empirical calculations using MNDO [27] report a corresponding value of 86.27°. The discrepancy is understandable, given the small differences in energy obtained by rotation about C4-C1′ (see Fig. 3). X-ray studies of MPTP yielded a value of 8° for this dihedral angle [28,29]. However, the solid-state data probably suffer from crystal-packing influences similar to those which have been reported for biphenyl [30]. In the X-ray studies of MPTP, crystal-packing influences apparently distorted the molecule such that some sp^2-sp^2 bonds were longer than sp^2-sp^3 bonds. Furthermore, a 21° deviation from sp^2 geometry of the angles around C4 was reported [28]. For MPTP, the calculated heat of formation is 39.21 kcal/mol, a value comparable to that reported earlier [27].

Substituent effects

In contrast to the saturated 1-methyl-4-phenylpiperidine, MPTP is a good substrate for MAO. The primary product of this enzyme-catalyzed oxidation of MPTP is the cationic azadiene $MPDP^+$. The observed rate enhancement suggests a role for the allylic double bond in the oxidation of MPTP. Consequently, the possibility that electronic effects transmitted through the phenyl ring to the vinyl group may influence the rate of oxidation was investigated by studying the net atomic charge at C5. The high electronegativity of fluorine and oxygen is clearly evident in the net positive charge on the phenyl carbon bearing these substituents. However, this electron-withdrawing effect is not fully transmitted to the tetrahydropyridine, as indicated by the net atomic charges at C5. On the whole, the net atomic charge at C5 is more negative for alkyl-substituted than for the corresponding halo-substituted analogs, a clear indication of the electron-withdrawing nature of the halogens (See Table 1). A chlorine atom at C2′ exerts significant electron withdrawal at C5, while the 2′-methoxy group is electron-releasing at C5. Substituents at C2′ are presumed to exert a significant inductive influence at C5 in view of their proximity to the vinyl group.

TABLE 1

BIOLOGICAL AND QUANTUM-MECHANICS-DERIVED DATA ON MPTP ANALOGS

Compound	Relative activity[a]		Net atomic charge C5	Twist[b] angle	C-2′/3′/4′[c] Net charge	Sum dipole	ΔH_f (kcal)	Ionization potential
	MAO A	MAO B						
MPTP	100	100	−0.1605	49.2	−0.1200	0.986	39.21	8.86988
2′-Me	(834) 417	244 (488)	−0.1615	106.6	−0.0531	0.822	33.44	8.99051
2′-Et	(966) 483	56 (112)	−0.1599	80.7	−0.0555	1.192	27.88	9.03216
2′-OMe	(716) 358	44 (88)	−0.1678	58.7	+0.0897	1.956	3.63	8.70559
2′-Cl	(558) 279	258 (516)	−0.1482	81.3	−0.0505	2.030	34.14	9.05266
2′-i-Pr	(1592) 796	10 (20)	−0.1609	77.1	−0.0515	1.194	23.94	9.01732
2-n-Pr	(916) 458	17 (34)	−0.1601	89.1	−0.0545	1.151	21.95	9.05757
2′,6′-Me_2	346	39	−0.1640	90.1	−0.0507	1.056	27.26	9.04832
3′-Me	54	124	−0.1612	130.5	−0.0702	0.844	31.67	8.83260
3′-F	275	173	−0.1528	49.7	+0.0887	1.584	−5.95	9.03526
3′-Cl	400	217	−0.1542	49.8	−0.0612	1.355	32.07	9.01672
3′-Br	212	390	−0.1542	50.1	−0.1665	1.458	44.01	9.03369
3′-OMe	0	180	−0.1563	33.9	+0.0779	1.641	1.32	8.78173
4′-Me	42	66	−0.1620	131.1	−0.0686	0.923	31.57	8.75260
4′-Cl	50	115	−0.1552	49.3	−0.0620	1.915	31.96	8.94331
4′-F	0	80	−0.1569	49.3	+0.0889	2.126	−6.13	8.92128
EPTP	21	39	−0.1587	38.6	−0.1297	0.985	33.84	8.78046
4-homo-MPTP	71	512	−0.1732	–	−0.1238	1.104	33.44	9.01940
MCTP	192	132	−0.0376	48.6	−0.1505	0.991	−18.22	8.96017
PPTP	51	6	−0.1601	50.0	−0.1202	0.949	27.31	8.84897

[a] Relative activity is the parameter. Turnover number/k_m (Ref. [26]) normalized to MPTP. Numbers in parentheses are based on the assumption that one atropisomer (or 50% of the sample) is inactive.

[b] Twist angle is defined as the dihedral angle C3-C4-C1′-C2′ for 4-homo-MPTP, the dihedral angle is C3-C4-C8-C1′. The sum dipole, twist angle, net atomic charge, heat of formation (ΔH_f) and ionization potential were obtained from semiempirical molecular orbital calculations.

[c] Net atomic charge at C2′ or the carbon bearing the substituent.

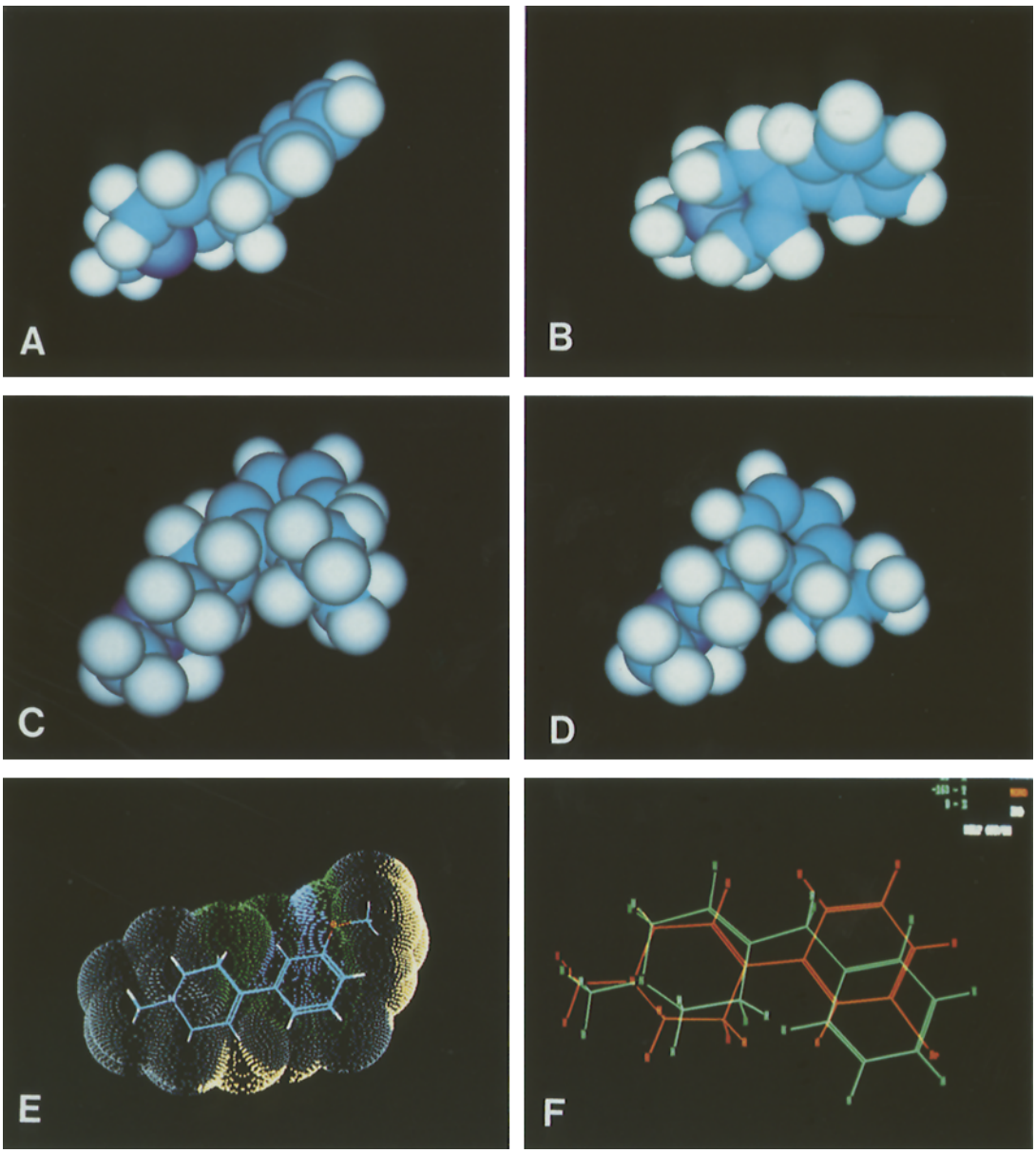

Fig. 2. (A) and (B) MOPAC-minimized space-filling models of MPTP showing the dihedral angle between the rings. (C) Space-filling model of the minimized structure of 2′-n-propyl-MPTP. (D) Space-filling model of the structure of 2′-i-propyl-MPTP. Note the different steric demands of the n-propyl and the i-propyl groups. (E) Minimized structure of 3′-methoxy-MPTP showing the molecular electrostatic potential distribution 2 Å from the surface of the molecule. Note that this analog is inactive in the MAO A assay. (F) Superposition of 4-homo-MPTP and 3′-bromo-MPTP. Note that the phenyl group of 4-homo-MPTP overlays the 3′-bromo substituent, and would therefore fit into the P3′-B pocket.

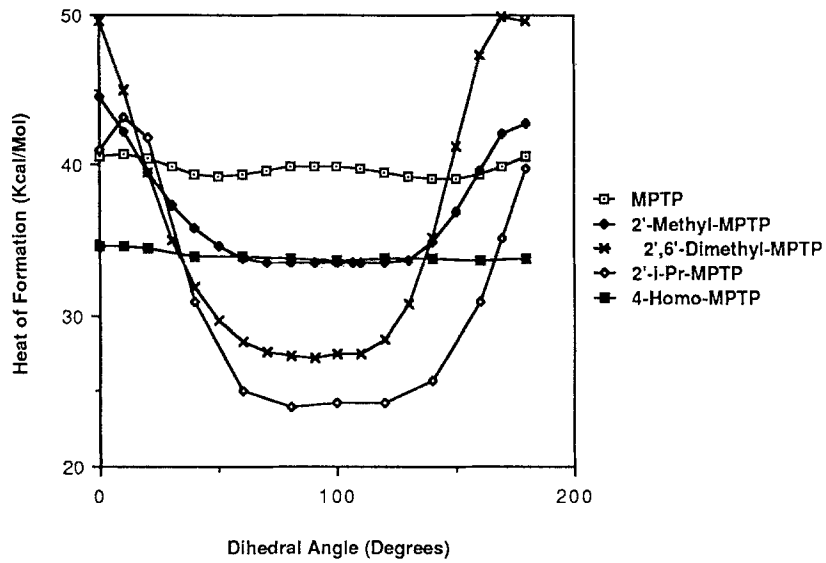

Fig. 3. Conformational-energy plot of MPTP and its analogs. Calculated heats of formation for a given dihedral angle plotted against the dihedral angle. The plots show broad regions of relatively free rotation flanked by high barriers for the 2′-substituted analogs.

However, this inductive influence may be offset, as in the case of the 2′-methoxy analog, by resonance effects. Thus, the discrepancy between the effects of the chlorine and methoxy groups at C2′ may be attributed to the extent of interaction between the rings. This interaction should approach zero as the ring-ring dihedral approaches 90°.

Although ring substitution causes variations in the net atomic charge at C5, the dipole moment and ionization potential, there is no obvious correlation between these parameters and substrate oxidative activity.

For both MAO A and B, the optimum substrate length measured along the main axis (N1-C4-C1′-C4′) of the molecules is about 8.5 Å. This measurement represents the distance between C4′ and the carbon atom of the N-methyl group. Alkyl-substitution at either end of this main axis results in significant reduction in oxidative activity (Table 1). However, the higher activity of the N-propyl analog over its N-ethyl congener may indicate greater bulk tolerance at this region of the MAO A substrate binding site. Although C4′-halogens cause only minor changes in oxidative activity for MAO B, significant reductions are observed for MAO A, suggesting greatly reduced tolerance for C4′-substitution by the latter isozyme. In any case, the length of the substrate binding site along this main axis is fairly well defined. As demonstrated by 4-homo-MPTP, significant increases in activity can be obtained by modifications which bend the molecule. The phenyl group in 4-homo-MPTP deviates from this main axis by 71°, and is therefore useful in defining another region of bulk tolerance within the substrate binding site. Finally, replacement of the phenyl group with the flexible cyclohexyl moiety (MCTP) yields an analog that is as good a substrate as MPTP.

Conformational studies

Conformational-energy plots derived from heats of formation at several dihedral angles indi-

cate that, for MPTP, 4-homo-MPTP (Fig. 3) and those analogs containing C4′ and C3′ substituents (data not shown), the barrier to rotation about the C4-C1′ bond is less than 2 kcal/mol. Thus, for all practical purposes, rotation about this bond is relatively unhindered. However, substitution at C2′ results in a significant barrier to rotation about the C4-C1′ bond. This barrier increases precipitously from the 2′-methyl (11 kcal/mol) through the 2′-isopropyl (16 kcal/mol) to the 2′,6′-dimethyl analog (22.3 kcal/mol). However, for these 2′-substituted analogs, two broad regions of relatively facile rotation (defined by the flat portions of the graph and its mirror image) about C4-C1′ are also evident (Fig. 3). Given these conformational restrictions, there would be minimal interaction between the pi systems of the phenyl and tetrahydropyridyl moieties. Therefore, a representation of these 2′-substituted compounds as extended pi systems would be less than accurate. Given these high rotational barriers, and the absence of symmetry along the main axis (N1-C4-C1′-C4′) of the molecule, the C2′-substituents remain locked on either side of the tetrahydropyridine, thereby producing an optically active molecule. Consequently, two atropisomers are produced.

DISCUSSION

The cyclic allylamine MPTP is rapidly oxidized by MAO. The primary product of this oxidation is the resonance-stabilized cation $MPDP^+$ which is rapidly oxidized to the stable MPP^+ [39,40]. In contrast to MPTP, 1-methyl-4-phenylpiperidine, the product of the partial hydrogenation of MPTP, is a poor substrate of MAO [41]. This contrast suggests that the allylic double bond greatly accelerates substrate oxidation. This acceleration may be attributed partly to a greater driving force leading to the resonance-stabilized cation $MPDP^+$. Consequently, the double bond is considered to be an important attribute of this class of compounds. Therefore, 1-methyl-4-phenylpiperidine, lacking this important attribute, is not considered a true MPTP analog within the context of this study.

From the results of these computational studies, there is no obvious relationship between the rate of oxidation and the molecular descriptors examined (e.g., dipole moment, ionization potential, dihedral angle, etc.). It is, nevertheless, clear that ring substitution and/or other structural modification affects both the rate of oxidation and the substrate specificity of the two isozymes, MAO A and MAO B. The foregoing observations suggest that other factors may be involved. Some insight into these factors may be obtained from further scrutiny of the conformational data. In this analysis, we have used a 'rigid active analog' approach, which presumes that a rigid active analog of the parent molecule closely approximates the stereoelectronic requirements for optimum ligand-receptor interaction, and therefore provides a suitable reference. A 'conformationally restricted analog', 2′-MeMPTP (see Fig. 1), has been chosen as the reference compound. Since the tetrahydropyridyl moiety is common to all analogs, only the effects of C4 substitution are considered. Furthermore, it is assumed that the alignment of the tetrahydropyridyl residue within the active site is essentially invariant; based on this assumption, the oxidative activity of chiral analogs (the C2′ atropisomers) would probably reside in only one isomer (vide infra). Consequently, the k_m values reported earlier should be halved, in order to approach the true values. When this is done, the Turnover number/k_m values double (see Table 1), and a reordering of activities becomes apparent. It also becomes clear that the relative molar potency of some of these analogs reported earlier by other workers may have been underestimated. The new numbers predict that 2′-

MeMPTP would be processed approximately five times more efficiently than MPTP. In this connection, it is worth noting that (1) 2′-MeMPTP is approximately five times more toxic than MPTP in vivo [19], and (2) following the administration of equimolar quantities of 2′-MeMPTP and MPTP, the peak brain concentration of 2′- $MeMPP^+$ is five times greater than that of MPP^+ [32]. This is in spite of the fact that 2′-$MeMPP^+$ is only slightly better than MPP^+ as a substrate for the dopamine reuptake [25], and 2′-$MeMPP^+$ and MPP^+ are essentially equipotent inhibitors of mitochondrial respiration [31].

For MAO B, it is clear that 2′-MeMPTP, 2′-chloro-MPTP and 4-homo-MPTP are essentially equivalent in activity, and 3′-bromo-MPTP is slightly less active. All other analogs are at least half as active as these four analogs. In general, the effects of substitution at C2′ are more pronounced in MAO A than MAO B.

As shown in Fig. 3, MPTP exhibits little or no barrier to rotation, and may therefore assume all the low-energy conformations of 2′-MeMPTP. On the other hand, 2′-MeMPTP and other 2′-substituted analogs may adopt several essentially isoenergetic minimum-energy conformations, but these are limited to dihedral angles ranging from 45° to 135° (and 225° to 315°). A recent study [21] suggests that the increased oxidative activity of 2′-MeMPTP relative to MPTP may be due to the destabilizing effect of the 2′-methyl substituent. As seen from Fig. 3 and Table 1, the 2′-methyl group causes an increased deviation from coplanarity. Using the calculated heats of formation as an index of stability, it is clear that with a ring-ring dihedral of 0° 2′-MeMPTP would be much less stable than MPTP. However, with a dihedral angle of 49.2°, 2′-MeMPTP is in fact more stable than MPTP. On the other hand, the calculated heats of formation for MPTP at 0° and 49.2° show little difference, indicating that coplanarity for this molecule affords little in the way of additional stabilization. Although this last conclusion would appear to run counter to classical resonance theory, one should note that resonance stabilization in this system may be opposed by other forces resulting from unfavorable steric interaction between the rings. In fact, as indicated by the activity of the cyclohexyl and benzylic analogs, an aromatic C4-substituent (see Table 1) is not required for oxidation by MAO. In an earlier study of MPTP analogs, Gibb et al. [33] also concluded that an aromatic C4 substituent was not necessary for MAO-catalyzed oxidation. This feature is the exact opposite of that reported by Knoll et al. [34] for a series of substituted propargylamines; in this instance, loss of aromaticity resulted in a drastic decrease in suicide-substrate inhibitor activity. These disparate structure-activity trends suggest that, for MAO, factors which determine substrate oxidative activity may be different from those which determine mechanism-based enzyme inhibition. Alternatively, these disparities may simply reflect the structural differences between the acyclic propargylamines and the conformationally restricted tetrahydropyridines. In view of the fact that 2′-MeMPTP is a better MAO substrate than MPTP, inability to assume a coplanar geometry would in itself appear to have little bearing on substrate oxidative activity. Since both 2′-MeMPTP and MPTP would exist preferentially in their minimum-energy conformations (with ring-ring dihedral angles of at least 45°), the large difference in oxidative activity between these two analogs can be attributed to the 2′-methyl substituent. This observation suggests that nonbonded hydrophobic interactions in the vicinity of C2′ are important for binding and subsequent reactivity. Although the importance of steric bulk at C2′ is a common feature of both MAO A and MAO B, the magnitude of the effects is dissimilar. For MAO A, there is a 16-fold difference in oxidative activity between MPTP and its 2′-isopropyl analog. In addition, there appears to be a progressive increase in the oxidative activity with increasing steric bulk at C2′. In fact, as

pointed out previously [21], selectivity for MAO A increases with increasing steric bulk at C2′. However, in MAO B, substituents larger than ethyl are poorly tolerated. It is also worth noting that 2′,6′-dimethyl-MPTP is less active than its 2-carbon equivalent 2′-ethyl-MPTP and the lower homolog 2′-MeMPTP. Since 2,6-dimethyl-MPTP is symmetrical, this difference in activity suggests that the nonbonded hydrophobic interactions in the vicinity of C2′ are 'one-sided'; this conclusion explains our earlier assumption that oxidative activity of C2′-substituted MPTP analogs probably resides in only one atropisomer. However, the expression of weak oxidative activity by the other atropisomer is not precluded.

In theory, substitution at C3′ should also yield atropisomers. However, the relatively facile rotation of the phenyl group about C4-C1′ would permit rapid interconversion between these isomers. Therefore, in practice, the existence of these atropisomers may be neglected. Substitution at C3′ with a methyl group yields a poor substrate while the presence of a methoxy group at the same position results in a totally inactive analog. On the other hand, all the 3′-halo-substituted analogs are better substrates of MAO A than MPTP. Taken together, these observations suggest that electron-withdrawing substituents are preferred at C3′. The net atomic charges at C5 confirm the electron-withdrawing effect of the halogens (see Table 1). However, the total inactivity of 3′-methoxy-MPTP may suggest that a combination of steric and electronic effects is operating at this location. As such, 3′-chloro-MPTP would represent the optimum balance between these two influences in MAO A. The higher activity of 3′-fluoro-MPTP relative to MPTP may also indicate that, when steric bulk is not limiting, electronic effects predominate. In MAO B, electron-withdrawing substituents at C3′ also increase substrate effectiveness. This observation suggests that the electronic demands of both isozymes are similar in this region. However, the steric constraints appear to be different. For the halogenated analogs, there is a progressive increase in oxidative activity with increasing bulk. 3′-BrMPTP is, in fact, one of the best substrates of MAO B. While 3′-MeMPTP and 3′-OMeMPTP are poor substrates of MAO A, both compounds perform better than MPTP as substrates of MAO B, suggesting that MAO B tolerates larger substituents at C3′ than does MAO A.

In general, substitution at C4′ is poorly tolerated in MAO A. However, the total inactivity of 4′-fluoro-MPTP cannot be easily explained. On the other hand, MAO B tolerates substituents as large as chlorine at C4′. Thus, it would appear that the substrate binding site along the main axis (N1-C4-C1′-C4′) is slightly longer in MAO B than in MAO A. However, no large enhancements in activity result from 4′-substitution. Substantial reductions in activity are observed when the N1 substituent is larger than a methyl group (see Table 1). This suggests that bulk tolerance in this region is limited. However, the diminished activity of the N-desmethyl analog, PTP [38], suggests a minimum steric requirement at this location.

In their study, Youngster et al. [21] used the Selectivity Index, defined as (Turnover number/k_m)$_{MAO\ B}$/(Turnover number/k_m)$_{MAO\ A}$ as a measure of substrate specificity. A selectivity index of one indicates no preference for either isozyme. With this approach, the relative importance of nonbonded hydrophobic interactions at C2′ and C3′ becomes fairly clear. Selectivity for MAO A increases with increasing steric bulk at C2′, while selectivity for MAO B increases with increasing steric bulk at C3′. By superimposing separately all good substrates of MAO A and MAO B, two composites are generated. In this context, a good substrate should be at least 80% as active as MPTP. These composites, which define the backbone of two new analogs (see Fig. 4a,b), closely approximate the outer limits of tolerance for MAO A and MAO B. The combination of these

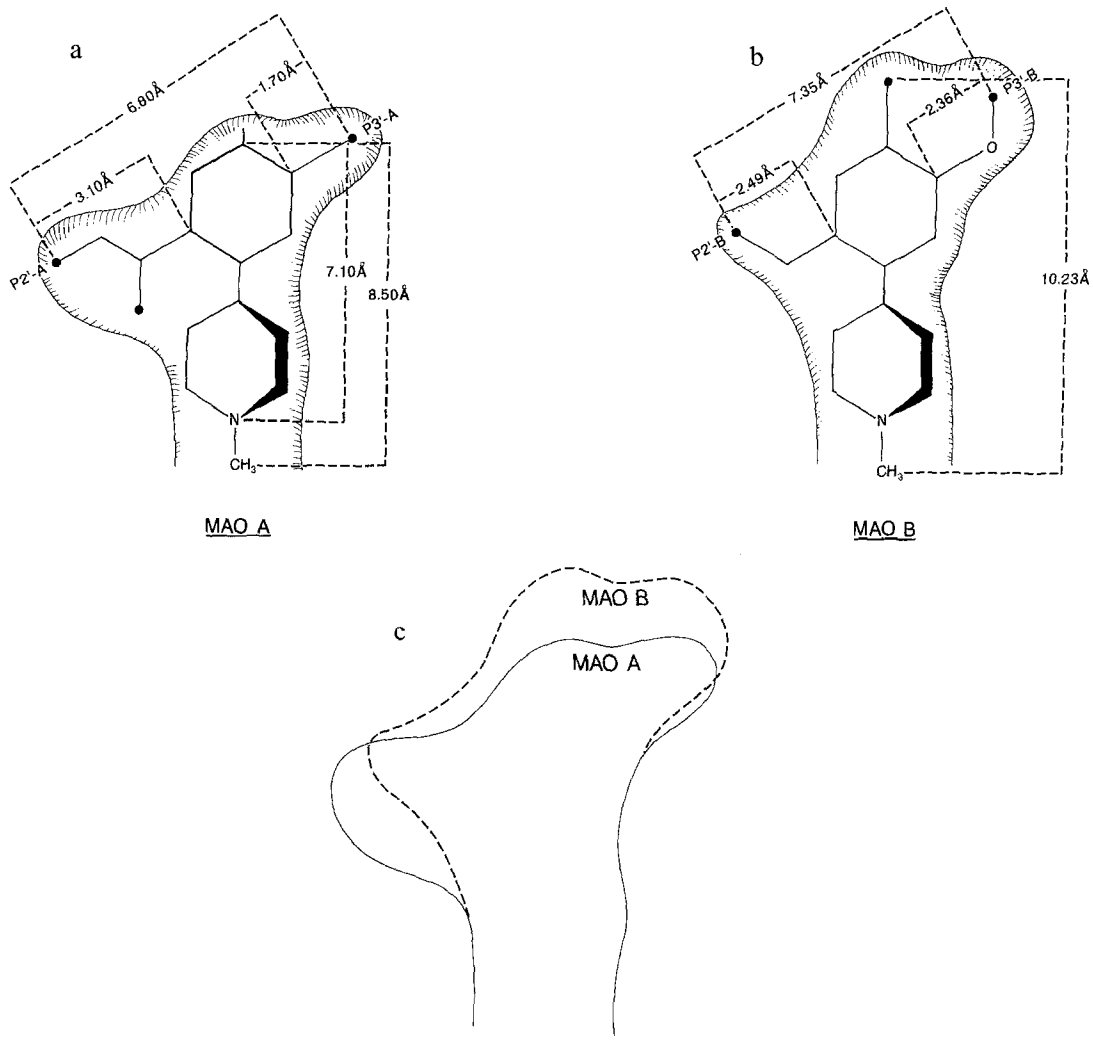

Fig. 4. Models of the substrate binding sites of MAO A (Fig. 4a) and MAO B (Fig. 4b). Within each binding site can be seen a composite which was derived by superimposing all good MPTP-like substrates of that isoenzyme. A good substrate is defined as having a Turnover number/k_m value no less than 80% that of MPTP. The dimensions given in the model were obtained from semiempirical molecular orbital calculations (MOPAC/AM1). Distances shown are those of the carbon skeleton indicated, and represent the maximum substituent tolerated at a given position on the phenyl ring. Note that the pocket P2'-A in MAO A is larger than P2'-B in MAO B. On the other hand P3'-B is larger than P3'-A. In Fig. 4c, the two substrate binding sites are superimposed to highlight the similarities and differences.

composites, the substituent effects discussed above and the conformational analysis forms the basis of a rudimentary model (see Fig. 4a,b) of the substrate binding sites of both MAO A and MAO B. The salient features of this model include:

(1) A substrate binding site which is approximately 8.5 Å long and is divided into two major subdivisions:

(i) the amine-binding region, and

(ii) the bulky substituent region.

The amine-binding region is a deep pocket approximately 2.5 Å wide (from C3 to C5), and it is proximal to the catalytic center of the enzyme. For optimum activity, the third substituent on the amine should be a methyl group.

(2) A hydrophobic pocket P2′ which accommodates substituents at the C2′ position of MPTP. In MAO A, this pocket (P2′-A) is large enough to accommodate an isopropyl or n-propyl group, while in MAO B a similar pocket (P2′-B) cannot accommodate a substituent larger than an ethyl group. Based on the conformational analysis, it is estimated that P2′ is oriented at least 45° away from the plane of the tetrahydropyridine moiety.

(3) A pocket P3′ which prefers lipophilic electron-withdrawing substituents at C3′. In MAO A, this pocket P3′-A is smaller than a similar pocket P3′-B found in MAO B.

This preliminary model has a number of limitations. Firstly, the orientation of P3′ relative to P2′ and the plane of the tetrahydropyridine is unknown. Such information may be obtained by studying the 2′,3′- and 2′,5′-disubstituted analogs. Secondly, the width of the 'bulky substituent' region is not known. However, preliminary evaluation of MNTP and MPHTP (see Fig. 1) suggests that the naphthyl group is well tolerated in this region, while a phenanthryl moiety is poorly tolerated [35]. These limitations notwithstanding, the present model can partly account for the substrate specificity of the two isozymes of MAO. A case in point is 4-homo-MPTP. The interposition of a methylene group between the phenyl and tetrahydropyridyl moieties results in a bent and flexible molecular skeleton, 4-homo-MPTP. This modification results in an excellent sub-

TABLE 2

CALCULATED (MOPAC/AM1) DIMENSIONS OF MPTP, ANALOGS OF MPTP AND OTHER ARYL (HETEROARYL) ALKYLAMINES

Molecule	Atom 1[a]	Atom 2[a]	Distance (Å)
MPTP	C4′	C7	8.52
	C2	C6	2.44
4-homo-MPTP	C7	C4′	7.44
Dopamine	N1	4′-OH	7.79
	N1	3′-OH	7.01
	C6′	3-OH′	4.17
Kynuramine	N1	C4′	6.52
	C5′	2′-NH_2	4.22
Serotonin	N1	C6′	7.88
	N1′	5′-OH	8.03
	C2′	5-OH′	5.94
MNTP	C2′	C6′	5.06
	C7	C7′	8.83
	N1	C7′	7.15
MPHTP	C3′	C8′	7.08

[a] Atom labels are shown in Fig. 1.

strate for MAO B (see Table 1). However, 4-homo-MPTP is a very poor substrate for MAO A. One explanation for this disparity may be obtained by a comparison of 4-homo-MPTP and 3′-bromo-MPTP (see Fig. 2F). The superpositions of these molecules clearly indicate that the phenyl group of 4-homo-MPTP extends into P3′-B. Given the larger size and electronic demands of this pocket in MAO B, the electron-rich phenyl group is apparently well suited to this region. However, the smaller pocket P3′-A in MAO A can not suitably accommodate the large phenyl group, thus the difference in oxidative activity. Although P3′ appears to prefer lipophilic groups, hydrophilic substituents may also be accommodated in this region. This view is supported by the reported MAO-catalyzed oxidation of 3′-hydroxy-MPTP [16].

The differences in these models explain, in part, the substrate specificities of these two isozymes. However, it is also clear that the similarities between the two models may explain why substrate selectivity manifests as a continuum rather than a discrete subdivision [36]. As shown in Table 2, the dimensions of serotonin, dopamine and kynuramine, known substrates of MAO, are such that they should fit into either binding-site model. If the 5-hydroxyl group of serotonin resides at P3′, then the 'pyrrole' fragment may be easily accommodated at P2′-A. Such a scenario would have P2′ and P3′ on opposite sides of the plane of the tetrahydropyridine ring.

In conclusion, a combination of conformational analysis and structure-activity data has been used to derive two rudimentary models which explain, in part, the substrate specificities of MAO A and B. These models may be useful in understanding the relative involvement of these two isozymes in the oxidation of biogenic and other amines. However, it is important to remember that the relative participation of these isozymes in vivo is a function of several factors, including substrate concentration, k_m, V_{max} and enzyme concentration.

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