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THEORETICAL STUDIES ON MECHANISM OF MPTP ACTION: ET INTERFERENCE BY MPP^+ (1-METHYL-4-PHENYLPYRIDINIUM) WITH MITOCHONDRIAL RESPIRATION *vs.* OXIDATIVE STRESS[1]

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This report demonstrates that ease of electron uptake by 1-methyl-4-phenylpyridinium (MPP^+), apparently the active agent derived from MPTP, is influenced by conformation of the phenyl ring. From quantum mechanical calculations on MPP^+, electron affinity is most negative for the nearly coplanar arrangement, indicating that the molecule is most readily reduced in this geometry. Ionization potential is largest in the perpendicular conformation, thus making for most facile oxidation in that form. Site binding would be expected to alter conformation in comparison with the situation in solution, and, hence, to influence reduction potential. We suggest that electron transfer by MPP^+ may play a role in inhibition of mitochondrial respiration and in oxidative stress.

KEY WORDS: MPTP, MPP^+, electron transfer (ET), inhibition of mitochondrial ET, oxidative stress, quantum mechanical calculations.

INTRODUCTION

In the early stages of mechanistic development, various lines of evidence indicated formation of activated oxygen species in the MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) (Figure 1) system.[1] Subsequently, there has been additional support for involvement of oxidative stress arising from MPTP, MPP^+ (1-methyl-4-phenylpyridinium) (Figure 2), or $MPDP^+$ (1-methyl-4-phenyl-2,3-dihydropyridinium).[2–8] Redox cycling by $MPDP^+$ appears attractive since its reduction potential is appreciably more positive than that of MPP^+.[8,9] Although some believe that $MPDP^+$ may be the actual neurotoxin,[10] its lifetime *in vivo* is not expected to be long due to ease of oxidation and the ability to generate potent oxidants *via* redox cycling. Most attention has centered on MPP^+ as the key oxidative metabolite derived from MPTP.[11–14]

Various investigators[4,7,8,14–20] concluded that MPP^+ does not act by an oxidative stress mechanism, based partly on the absence of activated oxygen products, ineffec-

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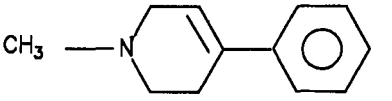

FIGURE 1 Structure of MPTP.

tiveness of antioxidants, and the rather negative reduction potential. In recent years increasing evidence has accumulated which indicates that the crucial effect may involve inhibition of mitochondrial energy production *via* interference with the electron transport system.[21-25] Mitochondria are vulnerable targets for damage by free radicals.[26,27] It is reasonable to hypothesize that agents which can participate in electron transfer (ET) might interfere with electron transport chains essential for mitochondrial respiration. Since quinones are well-known redox cycling agents, a good example is the anthraquinone derivative rhein which inhibits at the dehydrogenase coenzyme level *via* interference with ET.[28] We propose that MPP^+ may participate in an analogous manner by acting as an ET block or shunt while interfering with essential electron transport in the respiratory chain (eq. 1). Oxidative stress might also occur (eq. 2).

$$MPP + \overset{+e}{\rightleftharpoons} MPP^{\cdot} \tag{1}$$

$$MPP^{\cdot} + O_2 \rightarrow MPP^+ + O_2^{\cdot -} \tag{2}$$

This report deals with the influence of MPP^+ conformation on the ease of electron uptake. Site binding of the neurotoxin would be expected to alter conformation in comparison with the situation in solution, and, hence, to influence reduction potential. Quantum mechanical calculations are used to show the effect of conformational changes on energy, electron affinities and ionization potentials.

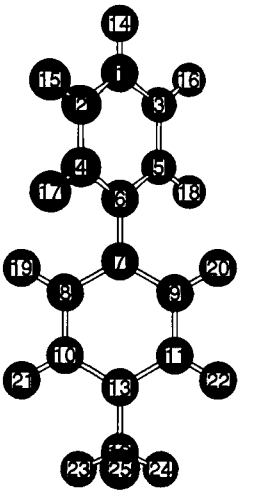

FIGURE 2 Structure of MPP^+.

METHODS

Molecular orbital calculations were the Intermediate Neglect of Differential Overlap (INDO) type using the scheme of Ridley and Zerner.[29,30] The atomic parameters used were the original INDO parameters of Pople and coworkers.[31] The geometry for the molecule was based on typical bond lengths for comparable systems[32] and assuming idealized bond angles. Torsional energy curves were obtained by rotating the phenyl ring relative to the pyridyl plane of MPP^+ (Figure 2) with all other coordinates frozen at the initially chosen geometry. The dihedral angle was changed in steps of 10 degrees. Ionization potentials (IP) and electron affinities (EA) were calculated by means of Koopmans' theorem[33] using the frozen orbital approximation. The calculations were run on a Hewlett-Packard 9000/350 workstation, a component of the Computational Facility for Theoretical Chemistry at the University of Idaho.

RESULTS AND DISCUSSION

Table 1 lists the Cartesian coordinates for MPP^+ with an inter-ring dihedral angle of 45 degrees. Table 2 gives the results of quantum mechanical calculations on this system as a function of dihedral angle; the data are summarized in Figures 3, 4 and 5. Figure 3 illustrates the plot of the total energy of the system as a function of dihedral angle. The results show the nearly coplanar system to be lower in energy by approximately 8 kcal/mole. Figure 4 provides the orbital energy for the highest occupied MO (HOMO) while Figure 5 shows the orbital energy for the lowest

TABLE 1
Cartesian coordinates for MPP^+ (in Angstroms) with an inter-ring dihedral angle of 45 degrees.

X	Y	Z	ATOM
0.000000	3.480000	0.000000	C
−0.849000	2.780000	0.849000	C
0.849000	2.780000	−0.849000	C
−0.849000	1.390000	0.849000	C
0.849000	1.390000	−0.849000	C
0.000000	0.695000	0.000000	C
0.000000	−0.695000	0.000000	C
−1.200000	−1.390000	0.000000	C
1.200000	−1.390000	0.000000	C
−1.200000	−2.780000	0.000000	C
1.200000	−2.780000	0.000000	C
0.000000	−4.910000	0.000000	C
0.000000	−3.480000	0.000000	N
0.000000	4.580000	0.000000	H
−1.520000	3.330000	1.520000	H
1.520000	3.330000	−1.520000	H
−1.520000	0.840000	1.520000	H
1.520000	0.840000	−1.520000	H
−2.150000	−0.840000	0.000000	H
2.150000	−0.840000	0.000000	H
−2.150000	−3.330000	0.000000	H
2.150000	−3.330000	0.000000	H
−0.898000	−5.280000	−0.581000	H
0.898000	−5.280000	−0.581000	H
0.000000	−5.280000	1.040000	H

TABLE 2
Relative Total Energies and Orbital Energies (in Hartrees) of MPP^+ Conformations.

Angle	Total Energy	Ionization Energy	Electron Affinity
10	−118.978841	−0.43382	−0.17830
20	−118.975825	−0.43395	−0.17796
30	−188.972771	−0.43397	−0.17744
40	−118.970374	−0.43374	−0.17681
50	−188.968565	−0.43319	−0.17617
60	−118.967206	−0.43238	−0.17563
70	−118.966256	−0.43151	−0.17523
80	−118.965698	−0.43085	−0.17499
90	−118.965518	−0.43060	−0.17491

unoccupied MO (LUMO). According to Koopmans' theorem, these orbital energies should correspond to experimentally measured IP's; EA's are experimentally difficult to measure and often have a large relaxation component. The figures demonstrate that as the phenyl fragment rotates out of the plane, the IP lowers slightly and then rises to a maximum at 90 degrees. The EA increases monotonically as the dihedral angle increases. Since this molecule is a cation, the EA is a negative number; therefore, the ion will accept an electron exothermically. The results lead to the conclusion that MPP^+ is most easily reduced in the nearly coplanar conformation and most readily oxidized in the perpendicular form. It should be noted that in all calculations the energy is for the unsolvated, gas-phase ion at absolute zero, and, hence, only approximates solution phase energy, Bodor *et al.* recently reported optimized geometry and stability for MPP^+ based on an MNDO study.[20]

A number of reports address the mode of binding by MPP^+ in the nigrostriatal neuronal system. There is no evidence for covalent attachment to intracellular protein.[34] It appears that site binding involves neuromelanin.[35-38] In solution the phenyl moiety of MPP^+ is relatively free to rotate. Conceivably, site binding hinders rotation because of steric constraints which may place the aryl group in a position more coplanar with the pyridinium ring. There is a relevant report of restriction of molecular motion *in vivo*.[39] Our calculations reveal that altered geometry could significant-

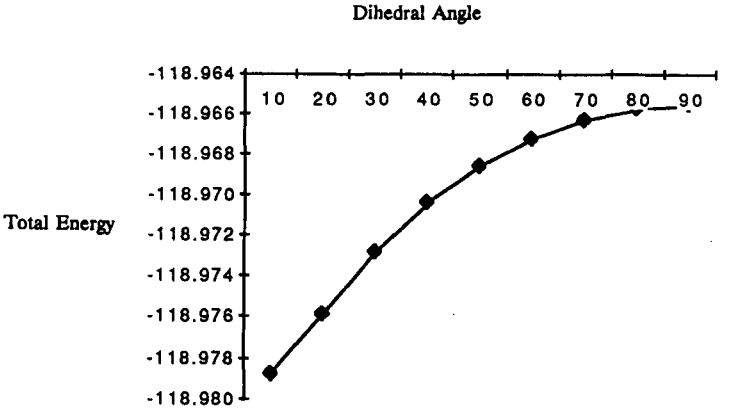

FIGURE 3 Total energy (in Hartrees) of MPP^+ *versus* inter-ring dihedral angle.

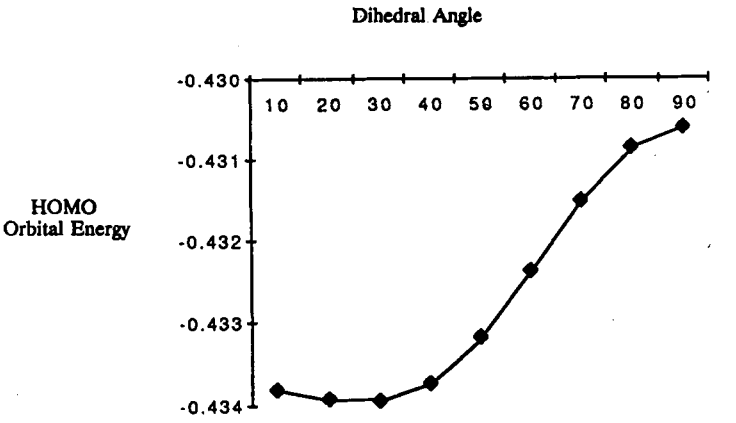

FIGURE 4 Ionization potential (in Hartrees) for MPP^+ *versus* inter-ring dihedral angle.

ly facilitate electron uptake and associated ET processes. The electrochemical characteristics of related model systems support this view. For example, the $E_{1/2}$ values for biphenyl and fluorene are -1.67 and -1.41 V (*vs.* NHE), respectively.[40] Since the methylene bridge of fluorene makes the $E_{1/2}$ value about 0.02 V more negative[41] (inductively), the coplanarity factor enhances the reduction potential by about 0.3 V, in general agreement with findings from the molecular orbital calculations for MPP^+ conformations. The reduction potential for MPP^+ in solution is about -0.9 V *vs.* NHE, converted from Ag/AgCl[9] and SCE[42] (cf. ref. 19). Hence, one would estimate a value of about -0.6 V for MPP^+ bound with nearly coplanar geometry *in vivo*, which may permit ET in the biological milieu.[17]

A recent review summarizes the current status for the mechanism of MPTP action.[43] Although oxidative stress by MPTP has been ruled out periodically by various investigators, reports pointing to formation of active oxygen species keep reoccurring, similar to resurrection of the proverbial cat. A recent example involves evidence for oxidative stress in the midbrain (but not in the striatum), including lipoperoxidation

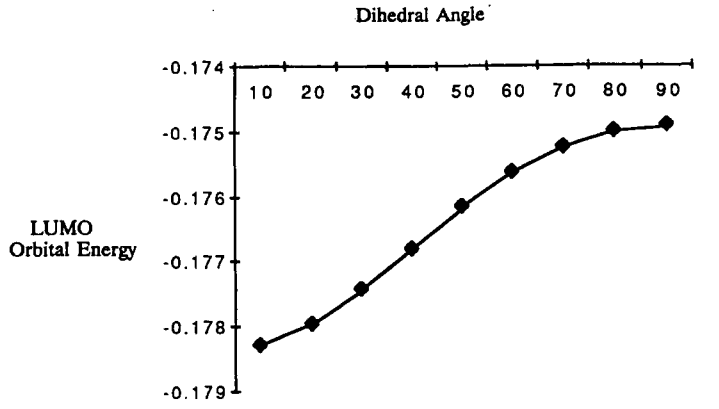

FIGURE 5 Electron affinity (in Hartrees) *versus* inter-ring dihedral angle.

when vitamin E is deficient.[44,45] From the totality of data it appears that oxidation can occur in certain brain areas, and under stringent conditions, in line with the marginal $E_{1/2}$ value of MPP^+. The mode of action most likely entails a component[43,46,47] based on inhibition of mitochondrial respiration, and an oxidative route[48,49] involving superoxide.

A similar situation involving influence of conformation on electroreduction may pertain in the case of phencyclidine. 1-(1-phenylcyclohexyl)piperidine (PCP). The derived iminium ion, generated by oxidative metabolism, reduced at about -0.7 V (NHE).[51] A recent conformational study of PCP revealed the occurrence of high affinity binding when the axial phenyl is oriented at an angle of 90°.[52] This geometry places the aromatic p-orbitals in the best position for stabilization by overlap with the electron formed by reduction of iminium. Hence $E_{1/2}$ should be increased. A related system comprises the nitroxide metabolite of cocaine, which apparently derives stabilization from through-space transannular effects during reduction.[53]

Acknowledgements

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