Communications

Cytotoxicity of 1-Amino-4-phenyl-1,2,3,6-tetrahydropyridine and 1-Amino-4-phenylpyridinium Ion, 1-Amino Analogues of MPTP and MPP^+, to Clonal Pheochromocytoma PC12 Cells

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1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) induces parkinsonism in humans after its oxidation into 1-methyl-4-phenylpyridinium ion (MPP^+) by type B monoamine oxidase. The 1-amino analogues of MPTP and MPP^+, 1-amino-4-phenyl-1,2,3,6-tetrahydropyridine (APTP) and 1-amino-4-phenylpyridinium ion (APP^+), were synthesized, and their cytotoxicity to clonal pheochromocytoma PC12 cells was examined using a tetrazolium formazan assay. After incubation for 48 and 72 h, both APP^+ and APTP were found to be cytotoxic to PC12 cells, whereas with the *N*-methyl analogues, only MPP^+, but not MPTP, was cytotoxic. The cytotoxicity of APTP increased with incubation time and equaled that of MPP^+ after 72 h. It was found that APTP was oxidized to APP^+ by type A monoamine oxidase in PC12 cells, suggesting that APP^+ itself may damage the cells. In addition to APTP and APP^+, *N*-amino analogues of *N*-methylisoquinolines and related derivatives were also synthesized and examined for their cytotoxicity to PC12 cells.

Introduction

Introducing an amino group at the ring nitrogen of heterocyclic compounds or replacing the *N*-methyl group of bioactive compounds with an *N*-amino group results in retention or enhancement of the biological activities, because the amino group can interact with surrouding macromolecules by hydrogen bonding. We have been working on the preparation of *N*-amino purine and pyrimidine derivarives and their biological activities (*1*−*5*). We reported previously that 3-aminothymidine inhibits the growth of CCRF leukemia cells more markedly than 3-methylthymidine (*1*). It was also reported that 3-amino-3′-azido-3′-deoxythymidine, a 3-amino derivative of 3′-azido-3′-deoxythymidine (AZT[1]), has better selective activity against HIV than AZT (*6*) and that 1-amino-5-halo-substituted uracil derivatives have anticonflict and anesthetic activities (*7*).

1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and its oxidation product, 1-methyl-4-phenylpyridinium ion (MPP^+), are well-known dopaminergic neurotoxins which cause parkinsonism in humans (*8*). Oxidation of MPTP by type B monoamine oxidase [amine oxidase, amine:oxygen oxidoreductase (deaminating), EC 1.4.3.4] (MAO-B) into MPP^+ is required for selective transport into dopamine neurons and cytotoxicity. The mechanism of the cytotoxicity is proposed to be the inhibition of ATP synthesis (*9*), or the induction of apoptosis (*10*). To examine the effect of the replacement of a methyl group with an amino group, 1-amino-4-phenyl-1,2,3,6-tetrahydropyridine (APTP) and 1-amino-4-phenylpyridinium ion (APP^+), the 1-amino analogues of MPTP and MPP^+, respectively, were synthesized and examined for their cytotoxic activity to PC12 cells using an XTT assay. The cytotoxicities of 2-amino analogues of other simple iso-

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[1]Abbreviations: AZT, 3′-azido-3′-deoxythymidine; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; MPP^+, 1-methyl-4-phenylpyridinium ion; APTP, 1-amino-4-phenyl-1,2,3,6-tetrahydropyridine; APP^+, 1-amino-4-phenylpyridinium ion; MAO-A, type A monoamine oxidase; MAO-B, type B monoamine oxidase; EPTP, 1-ethyl-4-phenyl-1,2,3,6-tetrahydropyridine; EPP^+, 1-ethyl-4-phenylpyridinium ion; $MAPP^+$, 1-(methylamino)-4-phenylpyridinium ion; MTIQ, 2-methyl-1,2,3,4-tetrahydroisoquinoline; MIQ^+, 2-methylisoquinolinium ion; ATIQ, 2-amino-1,2,3,4-tetrahydroisoquinoline; AIQ^+, 2-aminoisoquinolinium ion; DMEM, Dulbecco's Modified Eagle's Medium; PMS, phenazine methosulfate; MEM, minimal essential medium; XTT, 2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-2*H*-tetrazolium-5-carboxanilide.

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quinolines, 2′-methyl-1,2,3,4-tetrahydroisoquinoline (MTIQ), 2-methylisoquinolinium ion (MIQ^+), and related derivatives were also examined.

Experimental Procedures

Chemicals. MPTP hydrochloride, iodide salt of MPP^+, and 2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-2*H*-tetrazolium-5-carboxanilide (XTT) were purchased from Sigma (St. Louis, MO). Iodide salt of EPP^+ was from Aldrich (Milwaukee, WI). 2-Methyl-1,2,3,4-tetrahydroisoquinoline (MTIQ) hydrochloride and iodide salt of MIQ^+ were prepared as reported previously (*11*).

Syntheses of *N*-Amino Derivatives of Pyridines and Isoquinolines. General. ^1H and ^{13}C NMR spectra were recorded on a JEOL EX 270 or GSX 400 spectrometer (Tokyo, Japan), and chemical shifts are reported in parts per million (ppm) using tetramethylsilane as the internal standard. Mass spectra were obtained with a JEOL DX-300 spectrometer. pK_a values were determined with UV spectra using a Shimadzu UV-2100 spectrophotometer (Kyoto, Japan). Melting points were measured with a Yanagimoto micro-melting point apparatus (Kyoto, Japan) and are uncorrected. Silica gel 60 (Merck, 70−230 mesh, 63−200 μm) and silica gel PF254 (Merck) were used for column chromatography and preparative thin-layer chromatography (PLC), respectively.

1-Amino-4-phenylpyridinium Iodide (Iodide Salt of APP^+). 4-Phenylpyridine (6.21 g, 40 mmol) was added to a solution of hydroxylamine-*O*-sulfonic acid (1.50 g, 13.3 mmol) in 30 mL of water, and the mixture was heated at 90 °C for 2 h. After the mixture cooled to room temperature, water (30 mL) and K_2CO_3 (1.84 g, 13.3 mmol) were added. Precipitates were removed by filtration, and the mother liquor was washed with 40 mL of $CHCl_3$ three times. The aqueous phase was evaporated to dryness. EtOH (24 mL) was added to the residue, and the EtOH soluble fraction was collected by filtration. Concentrated HI (5.3 mL) was added to the filtrate, and the mixture was kept at −15 °C for 1 day. The dark brown needles that appeared were collected. The yield was 1.69 g (42.8%). Recrystallization from EtOH gave brown needles. Mp: 173−174 °C [lit. (*12*), 171 °C]. ^1H NMR (Me_2SO-d_6): δ 7.60−7.63 (m, 3H, Ph), 7.96−7.99 (m, 2H, Ph), 8.37 (d, 2H, J = 7.3 Hz, 3- and 5-H), 8.38 (s, 2H, NH_2), 8.80 (d, 2H, 2- and 6-H). ^{13}C NMR (Me_2SO-d_6): δ 124.7 (3- and 5-C), 127.5 (2′- and 6′-C), 129.5 (3′- and 5′-C), 131.3 (4′-C), 133.7 (1′-C), 138.5 (2- and 6-C), 149.5 (4-C). Anal. Calcd for $C_{11}H_{11}IN_2$: C, 44.31; H, 3.72; N, 9.40. Found: C, 43.99; H, 3.94; N, 9.57.

1-Amino-4-phenyl-1,2,3,6-tetrahydropyridine (APTP) Hydrochloride. Iodide salt of APP^+ (894 mg, 3 mmol) was dissolved in 50 mL of MeOH. $NaBH_4$ (227 mg, 6 mmol) was added, and the mixture was left at room temperature for 2 h. The product was purified by column chromatography (silica gel, 20 mm × 150 mm, eluted with $CHCl_3$ and then with 97:3 $CHCl_3$/MeOH). Fractions that contained product were collected, and the solvent was removed. To obtain the HCl salt, the residue was dissolved in 2 mL of 1 N HCl and the solvent was removed by evaporation. Recrystallization from MeOH/AcOEt gave crystals in a yield of 300 mg (57.5%). Mp: 185.1−186.4 °C. pK_a = 6.51. ^1H NMR (Me_2SO-d_6): δ 2.52 (t, 2H, J = 5.6 Hz, 3-H), 2.75 (t, 2H, 2-H), 3.18 (d, 2H, J = 2.9 Hz, 6-H), 6.08 (t, 1H, 5-H), 7.23 (t, 1H, J = 7.3 Hz, 4′-H), 7.32 (t, 2H, 3′- and 5′-H), 7.41 (d, 2H, J = 7.8 Hz, 2′- and 6′-H), 9.67 (br s, 2H, NH_2). ^{13}C NMR (Me_2SO-d_6): δ 27.9 (3-C), 55.8 and 58.3 (2- and 6-C), 121.6 (5-C), 124.5 (2′- and 6′-C), 126.9 (4′-C), 128.2 (3′- and 5′-C), 133.4 (4-C), 134.5 (1′-C). Anal. Calcd for $C_{11}H_{14}N_2$·HCl: C, 62.70; H, 7.18; N, 13.30. Found: C, 62.92; H, 7.06; N, 13.37. MS: *m*/*z* 174 (M^+ of free form).

1-Ethyl-4-phenyl-1,2,3,6-tetrahydropyridine (EPTP) Hydrochloride. Iodide salt of EPP^+ (50 mg, 0.16 mmol) was dissolved in 10 mL of MeOH. After $NaBH_4$ (25 mg, 0.66 mmol) was added, the mixture was left at room temperature for 10 min. The solvent was removed by evaporation, and the crude product was separated by PLC (silica gel, 9:1 $CHCl_3$/MeOH), yielding 30 mg. The product was solubilized in 10 mL of 10% HCl and evaporated to dryness. Recrystallization from AcOEt/MeOH gave colorless pillars. Mp: 206−207 °C [lit. (*13*), 192−194 °C]. ^1H NMR ($CDCl_3$): δ 1.18 (t, 3H, J = 7.3 Hz, CH_3), 2.56 (m, 2H, 3-H), 2.57 (q, 2H, CH_3CH_2), 2.74 (m, 2H, 2-H), 3.19 (d, 2H, J = 3.1 Hz, 6-H), 6.06 (br s, 1H, 5-H), 7.23 (t, 1H, J = 7.3 Hz, 4′-H), 7.31 (m, 2H, 3′- and 5′-H), 7.38 (d, 2H, J = 7.3 Hz, 2′- and 6′-H). Anal. Calcd for $C_{13}H_{17}N$·HCl: C, 69.78; H, 8.11; N, 6.26. Found: C, 69.62; H, 8.12; N, 6.40.

1-(Methylamino)-4-phenylpyridinium Iodide (Iodide Salt of $MAPP^+$). Acetic anhydride (3 mL) was added to iodide salt of APP^+ (500 mg, 1.54 mmol) in 2 mL of pyridine, and the mixture was left at room temperature for 15 h. After the solvent was removed by evaporation, the 1-(acetylamino)-4-phenylpyridinium salt was separated by PLC (silica gel, 9:1 $CHCl_3$/MeOH). The 1-(acetylamino)-4-phenylpyridinium salt obtained was dissolved in 5 mL of MeOH, and 0.5 mL of CH_3I was added. The mixture was left at room temperature for 2 days, and precipitates of 1-(*N*-acetyl-*N*-methylamino)-4-phenylpyridinium iodide were collected by filtration. The precipitates were dissolved in 5 mL of concentrated HCl, and the solution was heated at 100 °C for 1 h. After the solvent was removed by evaporation, the product was separated by PLC (silica gel, 4:1 $CHCl_3$/MeOH) to give iodide salt of $MAPP^+$ in a yield of 156 mg (29% yield starting from iodide salt of APP^+). Recrystallization from MeOH gave pale yellow pillars. Mp: 179−181 °C. ^1H NMR (Me_2SO-d_6): δ 3.11 (d, 3H, J = 4.5 Hz, CH_3), 7.66 (m, 3H, Ph), 8.01 (m, 2H, Ph), 8.42 (d, 2H, J = 6.8 Hz, 3- and 5-H), 8.58 (br, 1H, NH), 8.98 (d, 2H, 2- and 6-H). An analytically pure sample was not obtained after repeated recrystallization.

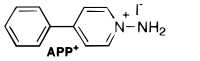
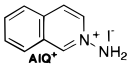
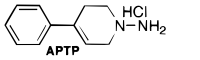
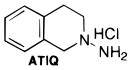
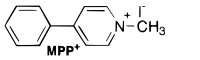
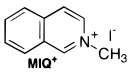
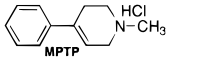
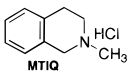
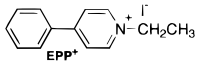
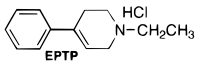
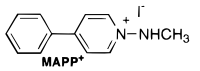
2-Aminoisoquinolinium Iodide (Iodide Salt of AIQ^+). Isoquinoline (387 mg, 3 mmol) was dissolved in 5 mL of DMF, and *O*-(2,4-dinitrophenyl)hydroxylamine (*14*) (900 mg, 4.5 mmol) was added. The mixture was heated at 60 °C for 15 h. After DMF was removed by evaporation, 5 mL of 1 N HCl was added, and the mixture was washed three times with 5 mL of AcOEt. The aqueous phase was evaporated to dryness, and the product was purified by column chromatography (silica gel, 30 mm × 200 mm, eluted with $CHCl_3$ and then with 12.5% MeOH in $CHCl_3$). The product was further purified by PLC (silica gel, 20% MeOH in $CHCl_3$). Recrystallization from MeOH/AcOEt gave yellow columns of 2-aminoisoquinolinium chloride in a yield of 424 mg (78.3%). Mp: 162−164 °C. ^1H NMR (Me_2SO-d_6): δ 8.02 and 8.13 (each t, each 1H, 6- and 7-H), 8.29 (d, 1H, J = 7.9 Hz, 4-H), 8.42 (d, 1H, 3-H), 8.50 and 8.57 (each d, each 1H, J = 7.3 Hz, 5- and 8-H), 8.86 (s, 2H, NH_2), 9.77 (s, 1H, NH). Anal. Calcd for $C_9H_9ClN_2$: C, 59.84; H, 5.02; N, 15.51. Found: C, 59.65; H, 5.28; N, 15.50.

Iodide salt of AIQ^+ was obtained by dissolving the chloride salt in aqueous hydriodic acid, followed by evaporation and recrystallization from MeOH/AcOEt. Mp: 177.5−178.5 °C [lit. (*15*), 180−183 °C]. Anal. Calcd for $C_9H_9IN_2$: C, 39.73; H, 3.33; N, 10.29. Found: C, 39.85; H, 3.36; N, 10.28.

2-Amino-1,2,3,4-tetrahydroisoquinoline (ATIQ) Hydrochloride. A procedure reported previously was followed (*16*). Briefly, the reaction of *o*-(β-chloroethyl)benzyl chloride (1.89 g, 10 mmol), which was prepared from isochroman (*17*), with benzoylhydrazine (1.63 g, 12 mmol) gave 2-(benzoylamino)-1,2,3,4-tetrahydroisoquinoline, and its subsequent acid hydrolysis yielded ATIQ hydrochloride in a yield of 472 mg (25.6%). Recrystallization from MeOH/AcOEt gave pale brown needles. Mp: 211−213 °C [lit. (*16*), 206−207 °C]. ^1H NMR (Me_2SO-d_6): δ 2.98 (t, 2H, J = 5.6 Hz, 4-H), 3.30 (t, 2H, 3-H), 4.15 (s, 2H, 1-H), 7.15−7.21 (m, 4H, 5-, 6-, 7-, and 8-H), 9.74 (br s, 2H, NH_2). Anal. Calcd for $C_9H_{12}N_2$·HCl: C, 58.53; H, 7.10; N, 15.17. Found: C, 58.50; H, 7.06; N, 14.88.

XTT Assay for Cytotoxicity. Fifty microliters of PC12 cells (4×10^5 cells/mL) in Dulbecco's Modified Eagle's Medium (DMEM) was seeded in each well of a 96-well plastic culture dish, and the cells were incubated for 1 day at 37 °C in 5% CO_2. The test chemical, dissolved in 50 μL of DMEM, was added to the cells, and the mixture was incubated at 37 °C for 24, 48, or

Table 1. Cytotoxicity of *N*-Amino and *N*-Methyl Derivatives and Related Compounds to PC12 Cells

Compound	IC_{50} (μM)[a]	Compound	IC_{50} (μM)[a]
 APP$^+$	123.7 ± 14.5	 AIQ$^+$	152.6 ± 4.7
 APTP	42.8 ± 3.3	 ATIQ	136.3 ± 28.9
 MPP$^+$	40.8 ± 8.9	 MIQ$^+$	278 ± 30.9
 MPTP	>1000	 MTIQ	>500
 EPP$^+$	72.6 ± 3.7		
 EPTP	>500		
 MAPP$^+$	200.9 ± 1.1		

[a] After incubation for 72 h.

72 h. The medium was then changed (100 μL), and the cells were incubated for another day. Fifty microliters of serum-free medium containing XTT (1 mg/mL) and phenazine methosulfate (PMS) (10 μM) was added to the cells which were then incubated at 37 °C for 4 h. The absorbance of XTT formazan at 450 nm was measured with a densitometer.

Formation of APP$^+$ from APTP. Homogenates of PC12 cells were prepared as follows. Cells (2×10^8) were collected by centrifugation and washed with PBS. The cells were then suspended in 4 mL of water and sonicated at 0 °C for 30 s (Handy Sonic UR 20-P, Tomy Seiko Co., Tokyo, Japan). The amount of protein of cell homogenates was determined by the method of Bradford using bovine γ-globulin as a standard (*18*). For the assay of APP$^+$ formation, 200 μL of 4 mM potassium phosphate buffer (pH 7.4) containing 0.38 or 1.25 mM APTP and increasing amounts of PC12 cell homogenate (0.10, 0.19, 0.48, 0.71, or 0.95 mg of protein) were incubated at 37 °C for 1 h. After the addition of 25 μL of 1 M perchloric acid containing 1 mM EDTA and 1 mM sodium metabisulfate, the mixture was centrifuged at 22000*g* for 10 min, and the supernatant was passed through a Millipore filter. The amount of APP$^+$ in the filtrate was determined with an HPLC system composed of an LC-9A pump and a spectrofluorometer (Shimadzu). A reversed-phase Inertosil ODS-2 column (4.6 mm × 250 mm, GL Sciences, Tokyo, Japan) was eluted at a flow rate of 1.0 mL/min with a solution consisting of 90 mM sodium acetate/35 mM citric acid buffer (pH 4.35), 130 μM EDTA, 100 μM 1-octanesulfonic acid sodium salt, and 23% methanol. The fluorescence at 370 nm was measured with excitation at 300 nm. For the experiments with enzyme inhibitors, several concentrations (final concentration of 1, 10, and 100 μM) of clorgyline (MAO-A inhibitor) or deprenyl (MAO-B inhibitor) were added to the mixture (200 μL) of APTP (1.25 mM) and cell homogenate (0.48 mg of protein).

Results and Discussion

Syntheses of *N*-Amino Derivatives of Pyridines and Isoquinolines. Structures of the test compounds employed in this study are shown in Table 1. Iodide salt of APP$^+$ was prepared by amination of 4-phenylpyridine with hydroxylamine-*O*-sulfonic acid. Since sufficient spectral data of iodide salt of APP$^+$ were not available (*12*), the results are presented in Experimental Procedures. Reduction of iodide salt of APP$^+$ with $NaBH_4$ yielded APTP. Similarly, EPTP hydrochloride (*13*) was prepared by reduction of iodide salt of EPP$^+$ with $NaBH_4$

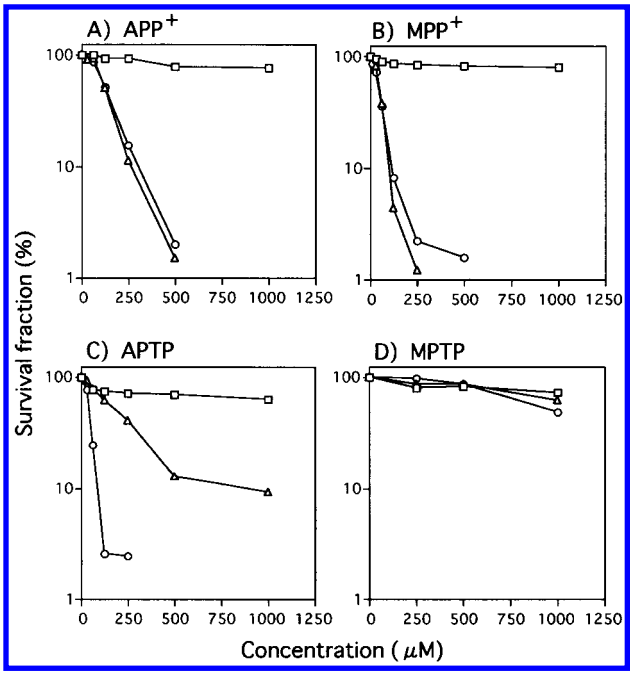


Figure 1. Cytotoxicity of APP$^+$, APTP, MPP$^+$, and MPTP to PC12 cells after treatment for 24 (□), 48 (△), and 72 h (○).

and fully characterized. Iodide salt of MAPP$^+$ was prepared by acetylation of APP$^+$, followed by methylation and deacetylation. We were unsuccessful in attempts to synthesize 1-(methylamino)-4-phenyl-1,2,3,6-tetrahydropyridine, the reduced form of MAPP$^+$, by the reduction of MAPP$^+$ with $NaBH_4$. Iodide salt of AIQ$^+$ (*15*) was prepared by amination of isoquinoline with *O*-(2,4-dinitrophenyl)hydroxylamine (*14*). ATIQ hydrochloride (*16*), iodide salt of MIQ$^+$ (*11*), and MTIQ hydrochloride (*11*) were prepared with reported procedures. Since sufficient spectral data of iodide salt of AIQ$^+$ and ATIQ hydrochloride were not available, these are also presented in Experimental Procedures.

Cytotoxicity of APTP and APP$^+$ to PC12 Cells. Cytotoxicity of APTP and APP$^+$ to PC12 cells was examined and compared to those of MPTP and MPP$^+$. The effect of the duration of treatment (24, 48, and 72 h) on cytotoxicity was tested. As shown in Figure 1A, treatment with APP$^+$ for 24 h had almost no effect, while after treatment for 48 h, there was a dose-dependent cytotoxicity. Treatment for 72 h did not increase cytotoxicity, indicating that full cytotoxicity is obtained after 48 h. A similar tendency was observed with MPP$^+$ (Figure 1B); however, MPP$^+$ had a stronger cytotoxicity than APP$^+$. IC_{50} values of MPP$^+$ and APP$^+$ at 72 h were 41 and 124 μM, respectively. For APTP, treatment for 24 h showed no cytotoxicity and treatment for 48 h showed only moderate cytotoxicity (Figure 1C). After 72 h, APTP showed strong cytotoxicity ($IC_{50} = 43$ μM), which was more than that of APP$^+$ and almost the same as that of MPP$^+$. MPTP showed only slight cytotoxicity even after 72 h (Figure 1D). The fact that APTP had strong cytotoxicity (Figure 1C) whereas MPTP very weak cytotoxicity (Figure 1D) is an indication of a big difference between the *N*-amino and *N*-methyl derivatives. PC12 cells primarily express the A but not the B type MAO (*19*), and MPTP is not a good substrate for MAO-A relative to MAO-B; thus, MPTP was not oxidized enough to be cytotoxic to PC12 cells. On the other hand, APTP was oxidized to APP$^+$ by the MAO-A of PC12 cells and

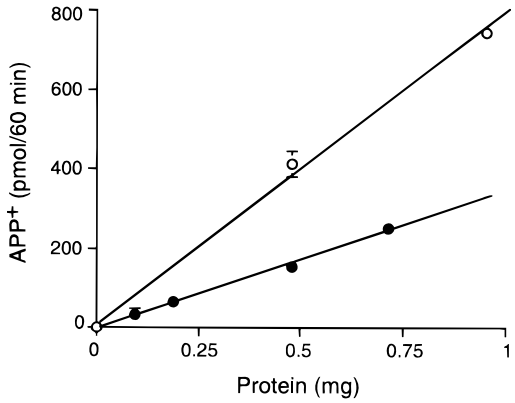


Figure 2. Formation of APP^+ from APTP in homogenates of PC12 cells. Two hundred microliters of 4 mM potassium phosphate buffer (pH 7.4) containing 0.38 or 1.25 mM APTP and increasing amounts of PC12 cell homogenate (0.10−0.95 mg of protein) was incubated at 37 °C for 1 h. The amount of APP^+ formed was quantitated by HPLC with a fluorospectrometer: 0.38 (●) and 1.25 mM APTP (○).

Table 2. Effect of MAO Inhibitors on the Formation of APP^+ from APTP by PC12 Cell Homogenates[a]

inhibitor	amount of APP^+ (pmol/60 min/mg protein)	yield[b]
none	506 ± 8	100
deprenyl (1 μM)	529 ± 2	105
deprenyl (10 μM)	528 ± 65	104
deprenyl (100 μM)	403 ± 3	80
clorgyline (1 μM)	236 ± 5	47
clorgyline (10 μM)	238 ± 74	47
clorgyline (100 μM)	59 ± 2	12

[a] Clorgyline (MAO-A inhibitor) or deprenyl (MAO-B inhibitor) was added to the reaction mixture (200 μL) containing APTP (1.25 mM) and cell homogenates (0.48 mg of protein). The mixture was incubated for 1 h, and the amount of APP^+ was quantitated as described in the legend of Figure 2. [b] Relative value.

showed strong cytotoxicity as described below.

Oxidation of APTP to APP^+ by Type A Monoamine Oxidase. To determine whether APTP is oxidized to APP^+ by MAO-A in PC12 cells, APTP was incubated with a homogenate of PC12 cells, and the amount of APP^+ formed was quantitated using an HPLC system equipped with a fluorospectrometer. As shown in Figure 2, the formation of APP^+ increased linearly with increasing amounts of cell homogenates. In the presence of the MAO-A inhibitor, clorgyline, the formation of APP^+ was inhibited (Table 2), while in the presence of the MAO-Binhibtor, deprenyl, no inhibiton was observed at concentrations of 1 and 10 μM, although a slight inhibition was seen at 100 μM. These results strongly indicate that APTP was oxidized to APP^+ by MAO-A in PC12 cells. The marked cytotoxicity of APTP is due to the efficient incorporation of APTP into the cells and its oxidation to the active form APP^+ by MAO-A. The fact that APTP requires much more time than APP^+ to show its full cytotoxicity (Figure 1A,C) supports this idea.

Cytotoxicity of *N*-Methyl and *N*-Amino Derivatives of Pyridines and Isoquinolines. Cytotoxicities to PC12 cells of 2-amino analogues of 2-methylisoquinoline derivatives and related compounds were examined. The IC_{50} values after treatment for 72 h are summarized in Table 1 together with those for APP^+, APTP, MPP^+, and MPTP. MPP^+ was 3 times more cytotoxic than APP^+ and 1.8 times more than EPP^+. When EPP^+ and $MAPP^+$ are compared, the former was 2.8 times more cytotoxic than the latter, and this ratio was almost the same as that for MPP^+ versus APP^+. Even with isoquinoline derivatives, ATIQ was cytotoxic as well as the AIQ^+ and MIQ^+, while MTIQ was not, as well as MPTP.

The mechanism proposed for the cytotoxicity of MPTP suggests that the MPP^+ formed reduces ATP synthesis via inhibition of Complex I (*9*) or α-ketoglutarate dehydrogenase (*20*). Also, the isoquinolines, MTIQ and MIQ^+, were reported to inhibit Complex I (*21*), but they were very weakly cytotoxic to PC 12 cells, as reported here and previously by others (*22*). On the other hand, our novel amino analogues of MPTP and MPP^+ were found to be much more cytotoxic than the isoquinolines. Although we have not examined whether APTP and APP^+ can inhibit Complex I activity in mitochondria, the reduction of ATP synthesis may be a mechanism for their cytotoxicity. In addition to the reduction of ATP, the hydroxy radical formation is proposed to be an another cytotoxic mechanism of MPTP (*23*). The radical formation is reported to result in induction of apoptosis in dopaminergic cells (*24*, *25*). Further investigation along this line with APTP and APP^+ is in progress.

In conclusion, APP^+, the 1-amino analogue of neurotoxic MPP^+, was cytotoxic to dopaminergic PC12 cells. APTP, a precursor of APP^+, was also cytotoxic to PC12 cells after it was oxidized to APP^+. This was different from the results with the corresponding 1-methyl analogue, MPTP, which was not cytotoxic to PC12 cells. This was due to the fact that the substrate specificity of MAO is different for APTP; i.e., APTP can be oxidized by both MAO-A and -B (*26*) while MPTP only by MAO-B.

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