[^{11}C]MPTP: A Potential Tracer for Parkinson's Disease Research in Laboratory Animals

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[^{11}C]-1-Methyl-4-phenyl-1,2,5,6-tetrahydropyridine ([^{11}C]MPTP), a compound producing parkinson-like symptoms in several species, has been synthesized and purified in sufficient activity to obtain tomographic images in the monkey. Biodistribution data has also been obtained in rats. Carbon-11-labeled MPTP could be used as a probe to study the pharmacokinetics of the compound under various research conditions in animals. Because of its neurotoxicity, the compound is not intended for use in humans.

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1 - Methyl - 4 - phenyl - 1,2,5,6 - tetrahydropyridine (MPTP) (**1**) is a by-product of the synthesis of a meperidine analog 1-methyl-4-phenyl-4-propionoxy piperidine (MPPP) (**2**) (*1*). The mixture (MPPP and MPTP) has sold as a street drug which produces severe neuropathies. MPTP is also thought to be responsible for inducing parkinsonism in humans (*2,3*). Furthermore, it has been shown to produce parkinson-like symptoms in monkeys (*4,5*) and selective degeneration of the nerve cells of the substantia nigra in mice (*6*). These observations have spurred a tremendous effort in the United States and Europe to characterize the mechanism of action of this drug, which in turn might give some insight into the natural course of the disease.

MPTP labeled with carbon-11 (^{11}C) or fluoro analog of MPTP labeled with fluorine-18 (^{18}F) both have the potential of elucidating the in vivo kinetic behavior of MPTP when used in conjunction with tomographic techniques. Furthermore, this tracer could be helpful in Parkinson's disease research in laboratory animals.

Accordingly, in the present study we have synthesized 1-[^{11}C]-methyl-4-phenyl-1,2,5,6-tetrahydropyridine ([^{11}C]MPTP) from 4-phenyl-1,2,5,6-tetrahydropyridine (PTP) (**3**). To study the in vivo behavior of this drug, we performed biodistribution studies in rats and imaged the monkey's brain using positron emission tomography (PET) following the i.v. administration of the ^{11}C-labeled drug.

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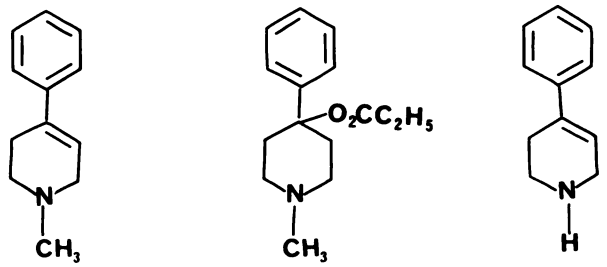

1 MPTP | **2** MPPP | **3** PTP

SCHEME 1

MATERIALS AND METHODS

Chromatography

Thin layer chromatography (TLC) was performed on silica gel 60 F 254* in methanol:chloroform: ammonium hydroxide (29.34% w/w), 10:90:1 (v/v/v). Chromatograms of radiolabeled compounds were scanned with a radiochromatogram scanner†. High performance liquid chromatography (HPLC) was carried out on a Partisil 5 SCX 10-cm column‡ with ethanol: 0.05*M* $NH_4H_2PO_4$, 60:40, at a flow rate of 2 ml/min.§

Chemistry

PTP. Obtained by neutralization of PTP.HCl¶. ^{1}H NMR ($CDCl_3$)**: 7.39 (m,5), 6.21 (m,1), 3.55 (d,J = 3Hz,2), 3.12 (t,J = 5Hz,2), 2.5 (m,2), 1.81 (S,1). Chemical purity was confirmed by TLC (Rf of PTP and

TABLE 1
Regional Brain Uptake of [^{11}C]MPTP in Rat

	% Dose/g*		
	5 min	15 min	30 min
Cerebellum	0.89 ± 0.17	0.52 ± 0.02	0.51 ± 0.08
Thalamus	1.10 ± 0.67	0.76 ± 0.14	0.49 ± 0.14
Striatum	1.34 ± 0.11	0.86 ± 0.13	0.56 ± 0.21
Cerebral cortex	1.14 ± 0.36	0.80 ± 0.05	0.51 ± 0.31
Remains	1.36 ± 0.26	0.86 ± 0.06	0.60 ± 0.13

* Four to six rats per time point.

PTP.HCl 0.14) and HPLC (>98%, retention time of PTP and PTP.HCl 2.88 min).

[^{11}C]*MPTP.* $^{11}CO_2$ was produced by deutron irradiation of boric oxide enriched with boron-10. The $^{11}CO_2$ was passed through a cold (0°C) solution of $LiAlH_4$ in ether** (0.5 ml, 1.0*M*). The ether was evaporated under a stream of He, and 0.4 ml HI solution (57%) was added. The solution was heated for 5 min at 140°C and the evolving $^{11}CH_3I$ was flushed with He into a cold (0°C) solution of PTP in acetonitrile (0.5 ml, 6 mg/ml). The solution was then heated at 65°C for 5 min and injected into the HPLC and the fraction corresponding to [^{11}C]MPTP was collected (2.2–3.3 mCi). The time required for synthesis and purification was 33–37 min (from EOB) and the yield was 5–7%. Specific activity of [^{11}C]MPTP (EOB) was 83.16–117.81 mCi/mmol. The radiochemical purity of [^{11}C]MPTP was ascertained by HPLC (>99%, retention time of [^{11}C]MPTP and MPTP†† 4.64 min) and TLC (>99%, Rf of [^{11}C] MPTP and MPTP 0.48).

Tissue Distribution Studies

CD Fischer rats (250–300 g) were anesthetized with ether and 0.2 ml (20–40 μCi) of [^{11}C]MPTP was injected through the tail vein. The rats were killed at 5, 15, and 30 min after administration of the dose. The appropriate organs and brain regions were excised and the radioactivity measured in a NaI (T1) well scintillation counter.

TABLE 2
Regional Brain Uptake of [^{11}C]MPTP in Rat

	% Dose/organ*		
	5 min	15 min	30 min
Cerebellum	0.35 ± 0.06	0.20 ± 0.02	0.20 ± 0.02
Thalamus	0.35 ± 0.05	0.26 ± 0.05	0.16 ± 0.04
Striatum	0.17 ± 0.04	0.09 ± 0.02	0.06 ± 0.03
Cerebral cortex	0.06 ± 0.03	0.06 ± 0.01	0.04 ± 0.03
Remains	1.17 ± 0.12	0.69 ± 0.01	0.47 ± 0.12

* Four to six rats per time point.

TABLE 3
Nonbrain Biodistribution of [^{11}C]MPTP in Rat

	% Dose/g*		
	5 min	15 min	30 min
Blood	0.23 ± 0.04	0.20 ± 0.01	0.13 ± 0.04
Heart	0.66 ± 0.11	0.57 ± 0.06	0.47 ± 0.08
Lung	1.62 ± 0.17	1.19 ± 0.16	0.91 ± 0.09
Liver	0.81 ± 0.29	1.09 ± 0.07	1.00 ± 0.20
Kidney	1.62 ± 1.55	1.61 ± 0.47	1.02 ± 0.46
Muscle	0.34 ± 0.12	0.24 ± 0.02	0.22 ± 0.06
Spleen	0.58 ± 0.22	0.46 ± 0.03	0.38 ± 0.11

* Four to six rats per time point.

Imaging of Monkey

PET imaging studies were carried out on pigtail monkeys anesthetized with pentobarbital. Each monkey's head was immobilized using a custom fabricated urethane cast. The monkey was then placed in the PC-384B PET Tomograph (*8*) and 1–2 mCi of the compound injected. Data for five tomographic planes were collected for 10-sec intervals during the first 8.33 min and for 1-min intervals during the subsequent 50 min. Quantitative reconstructions of the concentration of ^{11}C label at these time intervals were produced. Reconstruction of data summed over sequential 8-min epochs were also generated. The reconstructed images were 8 mm in plane resolution and 12 mm in thickness.

RESULTS AND DISCUSSION

Chemistry

The radiochemical yield of [^{11}C]MPTP was relatively low (5–7%) partially due to the production of a by-product that is thought to be 1,1-dimethyl-4-phenyl-1,2,5,6-tetrahydropyridinium iodide (DMPTP) labeled with ^{11}C. Our assumption of the presence of the by-product comes from the chromatography data. The compound stays at the origin under the conditions used for TLC and its elution time in HPLC is longer (9.71 min) than MPTP (4.64 min) and PTP (2.88 min). This

TABLE 4
Nonbrain Biodistribution of [^{11}C]MPTP in Rat

	% Dose/organ*		
	5 min	15 min	30 min
Blood	4.75 ± 0.15	4.12 ± 0.38	2.48 ± 0.93
Heart	0.68 ± 0.05	0.58 ± 0.03	0.43 ± 0.06
Lung	2.65 ± 0.47	1.89 ± 0.14	1.72 ± 0.88
Liver	9.21 ± 1.01	13.49 ± 0.43	10.30 ± 0.84
Kidney	3.27 ± 2.80	3.86 ± 1.31	2.08 ± 0.67
Muscle	40.92 ± 18.35	27.56 ± 1.32	24.62 ± 7.74
Spleen	0.47 ± 0.10	0.34 ± 0.11	0.28 ± 0.06

* Four to six rats per time point.

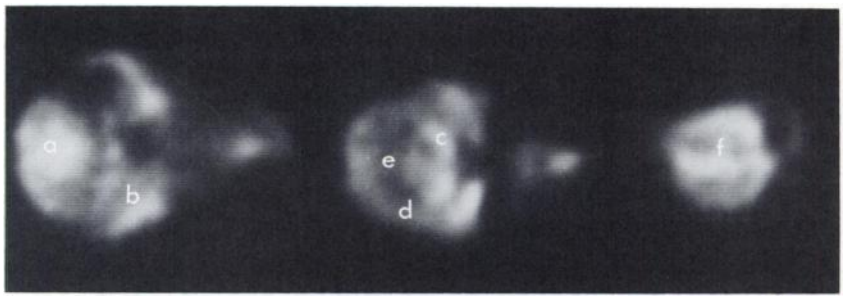

FIGURE 1
Tomographic images of monkey's brain showing a: cerebellum; b: temporal gray matter; c: right caudate; d: cortex; e: centrum semiovale; f: right hemisphere

order of elution is expected on a strong cation exchange column. The specific activity of [^{11}C]]MPTP is low (83.16–117.81 mCi/mmol) compared with the theoretical specific activity (9.2×10^9 mCi/mmol) due to the presence of methoxide in the $LiAlH_4$ solution.

Biodistribution Studies

Tables 1 and 2 contain the regional brain uptake in percent injected dose per g and percent injected dose per brain area.

The initial whole brain uptake was 2.09% injected dose per organ at 5 min and decreased to 1.30 and 0.93 at 15 and 30 min, respectively. The highest regional uptake in the brain in percent injected dose per g at all times was in the striatum (1.34 and 0.56 at 5 and 30 min, respectively). Wieczorek et al. (*7*) showed in an in vitro binding study that the highest [^{3}H]MPTP concentration bound in the rat brain occurred in the arcuate nucleus of the hypothalamus. These results indicate that [^{11}C]MPTP is extracted by the brain and that the activity decreases to ~50% between 5 and 30 min.

It is important to note that the cerebellum to striatum ratio decreases from 1.51 at 5 min to 1.09 at 30 min. This coincides with the fact that unlike mice, dogs, and monkeys (*9*), white rats are not affected by MPTP.

The biodistribution of [^{11}C]MPTP in other tissues of the rat is shown in Tables 3 and 4. The blood activity was 0.23% dose/g at 5 min and decreased to 0.13% at 30 min. At all times the highest uptake (% dose/organ) was in muscle followed by the liver.

Imaging Studies

Figure 1 presents three contiguous axial images of the head which were obtained parallel to the standard reference plane drawn from the inferior orbital ridge to the middle of the external auditory meatus. Figure 2 shows time (min) activity concentration (nCi/cc) curves taken over regions of interest in the three slices. The first plane, (left H_c − 08) through the posterior fossa-basal temporal lobe, shows the brain stem in the area of the pons and midbrain, the cerebellum, and the basal temporal lobe including some of the hippocampus and the amygdala. The activity in the cerebellum and the temporal gray matter reaches a maximum at ~15 min and remains at the same level for the rest of the study. The second axial image, (middle H_c + 06) is through the level of the basal ganglia, and includes the corpus striatum, (putamen and caudate), the thalamus, globus pallidus, and the internal capsule. The activity in the right caudate is higher at all times than that of the cortex and the centrum semiovale. The third image, (right H_c20) through the high ventricular region, shows the centrum semiovale as well as the cortical regions. The activity in the right and left hemisphere rises for the first 20 min and remains the same afterwards.

The average of the activity concentration (nCi/cc)

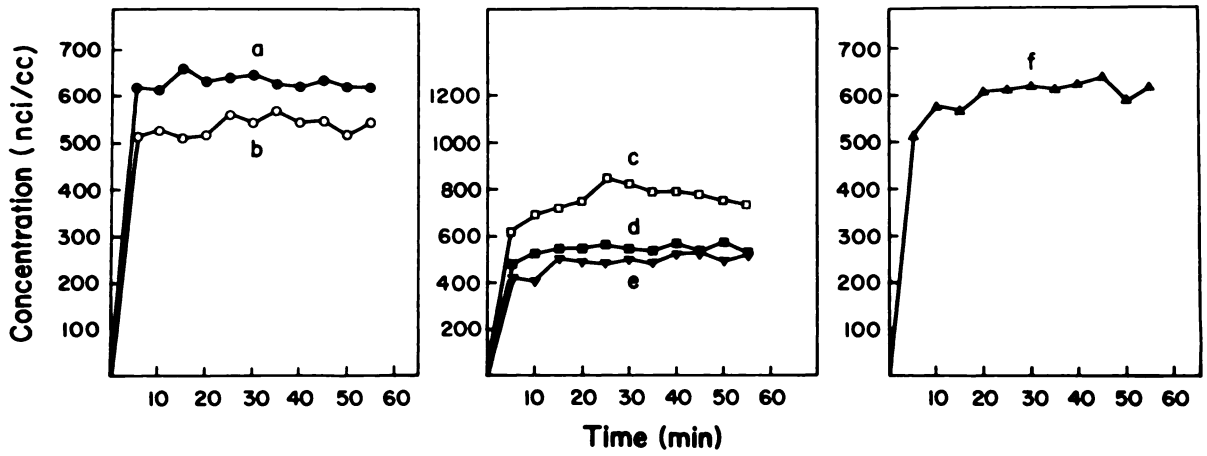

FIGURE 2
Radioactivity concentration as function of time for regions of interest labeled in Fig. 1

TABLE 5
Distribution of ^{11}C Radioactivity in Monkey's Brain

Region	Concentration (nCi/cc)*
Temporal gray	524 ± 52
Cerebellum	623 ± 24
Right cortex	550 ± 33
Posterior cortex	571 ± 16
Centrum semiovale	499 ± 42
Left caudate	732 ± 55
Right caudate	786 ± 49
Right hemisphere	549 ± 20
Left hemisphere	619 ± 17

* Mean ± s.d.

between 38.33 min and 58.33 min is presented in Table 5. The highest activity concentration is in the right and left caudate, and the lowest concentration of activity is in the centrum semiovale. Similar results were obtained from autoradiographic images by Markey et al. (*10*). The autoradiographic images showed high concentrations of radioactivity in the caudate nucleus and low levels in white matter.

CONCLUSIONS

Our preliminary results suggest that [^{11}C]MPTP can be produced and purified easily in sufficient amounts of activity to obtain tomographic images in the monkey. Because of its neurotoxicity, the compound is not intended for use in humans. However, with the availability of a monkey model for Parkinson's disease (*4*) MPTP labeled with ^{11}C could be used to study the pharmacokinetics of the compound before and after the administration of drugs used in Parkinson's disease research.

FOOTNOTES

* American Scientific Products, Boston, MA.
† Bioscan, Washington, DC.
‡ Whatman Inc, Clifton, NJ.
§ Laboratory Data Control, Riviera Beach, FL.
¶ Aldrich Chemical Company, Inc., Milwaukee, WI.
** Varian T-60 spectrometer with $(CH_3)_4Si$ as internal standard, Milwaukee, WI.
†† Research Biochemicals, Wayland, MA.

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