Kinetics of [^{11}C]N,N-Dimethylphenylethylamine in Mice and Humans: Potential for Measurement of Brain MAO-B Activity

Hitoshi Shinotoh, Osamu Inoue, Kazutoshi Suzuki, Toshiro Yamasaki, Masaomi Iyo, Kenji Hashimoto, Toshiyoshi Tominaga, Takashi Itoh, Yukio Tateno, and Hiroo Ikehira

Division of Clinical Research, National Institute of Radiological Sciences, Section of Cyclotron, National Institute of Radiological Sciences, Japan

Carbon-11-labeled N,N-dimethylphenylethylamine ([^{11}C]DMPEA) was synthesized by the reaction of N-methylphenylethylamine with [^{11}C]methyl iodide. This newly synthesized radiotracer was developed for the purpose of in vivo measurement of monoamine oxidase-B activity in the brain using a metabolic trapping method. Initially, biodistribution was investigated in mice. The rapid and high uptake of ^{11}C radioactivity in the brain was observed following intravenous injection of [^{11}C]DMPEA, the peak of which was reached at 1 min, followed by a decrease at 1–5 min and slowly thereafter. The kinetics of [^{11}C]DMPEA in the human brain were determined using positron emission tomography (PET) and showed that ^{11}C radioactivity increased gradually over 60 min following initial rapid uptake of ^{11}C radioactivity, with basal ganglia and thalamus showing high accumulation.

J Nucl Med 28:1006–1011, 1987

Monoamine oxidase (MAO) is widely distributed throughout the body and catalyzes the oxidative deamination of a variety of monoamines (*1*). MAO has been classified into two main types: MAO-A and MAO-B. MAO-A deaminates serotonin (5-HT) and noradrenaline (NA) much better than phenylethylamine (PEA) or benzylamine, and is preferentially inhibited by clorgyline, whereas, MAO-B prefers PEA and benzylamine as substrates and is preferentially inhibited by l-deprenyl (*2–4*).

Alterations of MAO-B activity in the brain has been implicated in aging, Alzheimer's disease, Huntington's disease, alcoholism, suicides, and affective illness (*5–11*).

If it were possible to measure MAO-B activity in the human brain by positron emission tomography (PET), it would offer valuable information about human brain function and neurochemical abnormalities in some neuropsychiatric disorders.

Recently, several attempts have been made to develop new tracers for in vivo estimation of brain MAO activity (*12–16*). One of our group (I.O.) developed labeled phenylethylamine derivatives as tracers for the study of the brain MAO activity using a metabolic trapping method (*14–16*). N-(methyl-^{14}C)N,N-dimethylphenylethylamine ([^{14}C]DMPEA) was found to be the most promising among them because of its high selectivity for MAO-B, its high brain uptake and its wide range of measurable brain MAO-B activity (*16*).

Kinetics of [^{14}C]DMPEA in mice have been reported as follows (*16*) (Fig. 1): [^{14}C]DMPEA enters the mouse brain rapidly following intravenous injection by first pass uptake because of its lipophilicity. A part of [^{14}C] DMPEA in the brain is deaminated selectively by the brain MAO-B. Its metabolite, ^{14}C-labeled dimethylamine, is trapped in the brain because its pKa value is very high (10.64) (*17*) and protonated dimethylamine cannot pass through the biologic membranes, including the blood–brain barrier. On the other hand, unmetabolized [^{14}C]DMPEA is eliminated from the brain rap-

Received Apr. 25, 1986; revision accepted Nov. 3, 1986.

For reprints contact: Hitoshi Shinotoh, MD, Div. of Clinical Research, National Institute of Radiological Sciences, 9-1 Anagawa-4-chome, Chiba-shi, CHIBA 260, Japan.

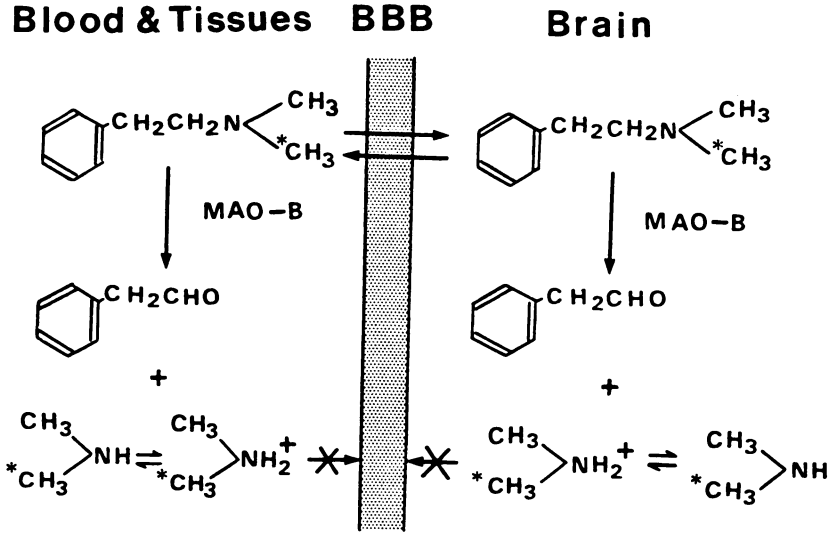

FIGURE 1
Proposed mechanism of metabolic trapping of labeled DMPEA in the brain. *: ^{14}C- or ^{11}C-labeled position. BBB: blood–brain barrier.

idly. Therefore, only [^{14}C]dimethylamine remains in the brain 60 min after injection. An inhibition experiment with l-deprenyl revealed that the initial uptake of [^{14}C] DMPEA was not altered but the clearance of ^{14}C radioactivity in the brain became faster as the dose of l-deprenyl pretreated was increased. [^{14}C]dimethylamine production in the mouse brain at 60 min after injection correlated well with remaining MAO-B activity after pretreatment with various doses of l-deprenyl.

These results indicate that relative MAO-B activity in the human brain could be measured by PET using ^{11}C-labeled DMPEA instead of [^{14}C]DMPEA.

In this report, we describe synthesis of [^{11}C]DMPEA, biodistribution of ^{11}C radioactivity, effects of MAO inhibitors on kinetics of ^{11}C radioactivity in mice following injection of [^{11}C]DMPEA, and kinetics of ^{11}C radioactivity in the human brain following injection of [^{11}C]DMPEA.

MATERIALS AND METHODS

Synthesis of [^{11}C]DMPEA

Carbon-11 was produced with 18 MeV protons from the NIRS cyclotron* by the ^{14}N (p, α)^{11}C reaction. The ^{11}C generated was converted to [^{11}C]CO_2 by passage through a CuO column at 800°C. Carbon-11 labeled methyl iodide was prepared from [^{11}C]CO_2 by reduction with $LiAlH_4$ and distillation of [^{11}C]methanol in a flow of nitrogen through refluxing concentrated hydroiodic acid. A mixture of 10–50 μl N-methylphenylethylamine,† [^{11}C]methyl iodide, and 5–20 μl 10 M NaOH in 0.5–1 ml of acetone was heated for 5 min at 70°C. The [^{11}C]DMPEA produced was purified by high-performance liquid chromatography with chloroform/methanol/ammonia (1500:50:1) as a solvent on a silica gel column (7.2 mm ϕ × 250 mm)‡ at a flow rate of 5 ml/min. The retention time of the product and the substrate were 4 and 10 min, respectively. The fraction containing [^{11}C]DMPEA was collected and evaporated. The residue was dissolved in 10 ml of saline and passed through a sterile Millipore filter (0.22 μm). The time of preparation was ~35 min from the end of bombardment. The yield of [^{11}C]DMPEA for intravenous injection varied between 13 and 90 mCi, the radiochemical yield was ~20%, and the radiochemical purity was >99%. The specific activity was >100 mCi/μmol estimated from the detection limit of an ultraviolet detector. The product was sterile and pyrogen free.

Biodistribution in Mice

Male C3H mice (12 wk old) weighing 33 g were used. Mice were injected via the tail vein with no-carrier-added [^{11}C] DMPEA (50 μCi/0.2 ml, <2.3 μg/kg). Mice were killed 1, 5, 15, 30, and 60 min following injection. Tissues were removed, washed with saline, and weighed. The radioactivity was measured with a sodium-iodide scintillation counter. The uptake was expressed as percentage dose administered per gram organ.

Effect of MAO Inhibitors

Male C3H mice (12 wk old) were pretreated with various doses (0, 0.01, 0.1, 10 mg/kg, i.v.) of l-deprenyl§ and a single dose of 10 mg/kg of clorgyline¶ 60 min before injection of [^{11}C]DMPEA. Mice were killed 60 min after injection of [^{11}C] DMPEA and the radioactivity of the brain was measured as described above.

Experiments with [^{11}C]DMPEA in Human Subjects

Four male volunteers, varying in age from 48 to 70 yr took part in the study. These subjects were healthy at the time of physical and laboratory examination. Informed written consent was obtained from the subjects.

A three-ring PET system ("Positologia II") (*18*) was used to obtain quantitative data on the regional radioactivity within a section of the brain. The spatial resolution of the reconstructed images is 9.2 mm full width at half maximum (FWHM). The slice thickness is 10 mm (FWHM) for cross slices and 13 mm (FWHM) for direct slices.

Each subject lay supine with his head positioned in the PET scanner, so that the lowest slice corresponded to 10 mm above the canthomeatal line. A venous cannula was inserted into an antecubital vein for isotope injection. A dose of 9.1 ± 2.9 mCi (mean ± s.d. <18 μg) of no-carrier-added [^{11}C]DMPEA was administered over a period of 10 sec.

Serial PET images (each scan took 1–2 min) were obtained

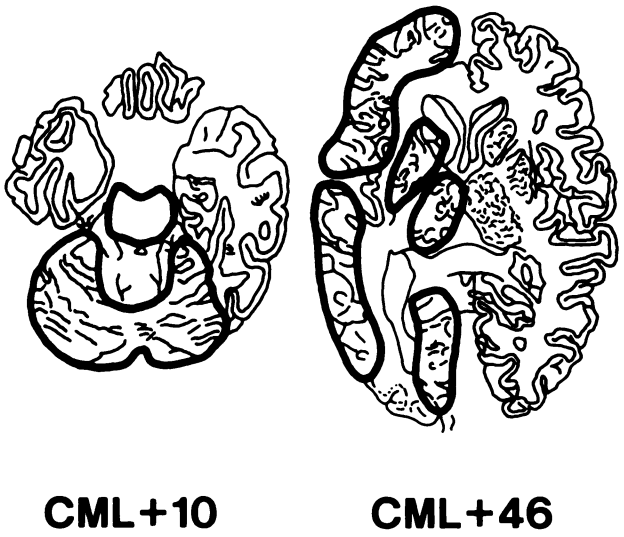

FIGURE 2
Regions of interest in the brain. ROI in the cerebrum were drawn in the left hemisphere.

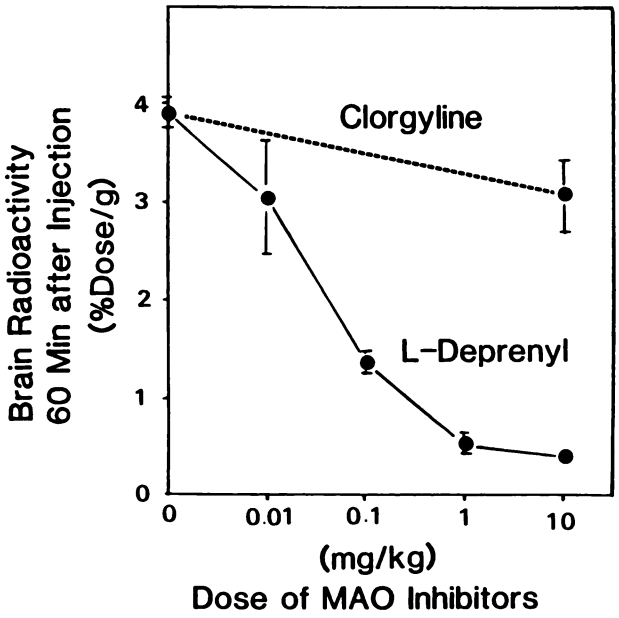

FIGURE 3
Effect of MAO inhibitors to the kinetics of [^{11}C]DMPEA in the mouse brain. The brain radioactivity 60 min after injection was expressed as percent dose administered per gram (mean ± s.d. of three mice).

for 30–60 min immediately following injection. Simultaneous venous blood samples were obtained at 1–10 min intervals during a period of 30–60 min, and the radioactivity in 1 ml of blood was measured with a sodium-iodide scintillation counter.

Regions of interest were the frontal cortex, temporal cortex, occipital cortex, basal ganglia (which included the caudate, putamen, and pallidum), thalamus, cerebellum, and brain stem (Fig. 2). Regional activity was measured for each sequential scan, corrected for ^{11}C decay, and plotted on a time basis. Partial volume effects were not corrected in this study. Regional activity and blood activity was expressed as percent injected dose per ml.

RESULTS

Biodistribution in Mice

Table 1 shows the biodistribution in mice. [^{11}C] DMPEA was well transported into many organs and was cleared rapidly from the blood. The highest uptake was observed in the kidney. A slightly lower uptake was observed in the brain and the lung. In the brain, the uptake of ^{11}C radioactivity reached its peak 1 min after injection, the clearance was rapid at 1–5 min and slow thereafter until the end of experiment. The activity in the blood decreased rapidly and reached a much lower level than that in the brain.

Effects of MAO Inhibitors

The radioactivity in the brain 60 min after injection decreased in a dose-dependent mode with pretreatment of various doses of l-deprenyl, whereas, the radioactivity in the blood was not altered significantly. The blood radioactivity 60 min after injection with pretreatment of 10 mg/kg of l-deprenyl was 0.4 ± 0.4 %dose/g (mean ± s.d. of three mice), whereas, that of control mice was

TABLE 1
Tissue Distribution of Radioactivity in Mice After Intravenous Injection of [^{11}C]DMPEA

Tissue	1 min	5 min	15 min	30 min	60 min
Brain	7.9 ± 0.4	5.1 ± 1.0	3.9 ± 0.4	3.5 ± 0.1	2.8 ± 0.3
Heart	5.4 ± 0.3	2.9 ± 0.3	1.5 ± 0.1	0.8 ± 0.1	0.4 ± 0.1
Lung	7.9 ± 0.6	4.3 ± 0.5	2.0 ± 0.1	1.3 ± 0.0	0.7 ± 0.2
Liver	3.2 ± 0.9	4.2 ± 0.7	2.1 ± 0.1	1.4 ± 0.1	0.8 ± 0.1
Spleen	2.1 ± 0.6	3.9 ± 0.7	3.9 ± 0.3	2.5 ± 0.1	1.4 ± 0.4
Kidney	11.3 ± 1.7	12.0 ± 2.1	9.4 ± 1.5	5.7 ± 0.1	4.0 ± 1.0
Small intestine	4.8 ± 1.0	4.5 ± 0.3	2.1 ± 0.1	1.5 ± 0.4	0.9 ± 0.1
Testis	1.5 ± 0.2	1.9 ± 0.5	1.7 ± 0.2	1.3 ± 0.1	1.0 ± 0.1
Muscle	3.7 ± 0.7	2.3 ± 0.2	1.7 ± 0.3	0.9 ± 0.1	0.4 ± 0.1
Blood	2.3 ± 0.3	1.9 ± 0.1	1.0 ± 0.1	0.5 ± 0.1	0.2 ± 0.1

Mean uptake ± s.d. (% dose/g) of three mice.

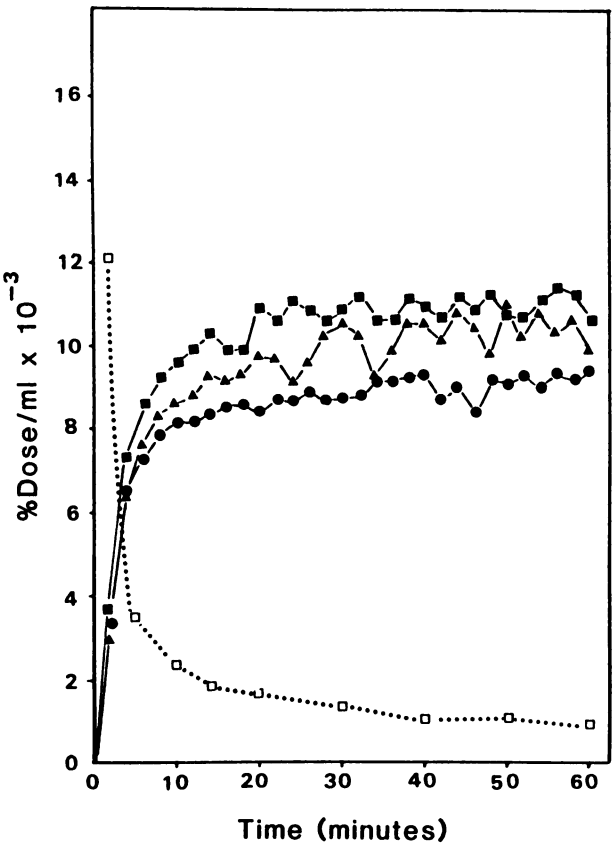

FIGURE 4
Kinetics of ^{11}C radioactivity in the human brain after intravenous injection of [^{11}C]DMPEA.

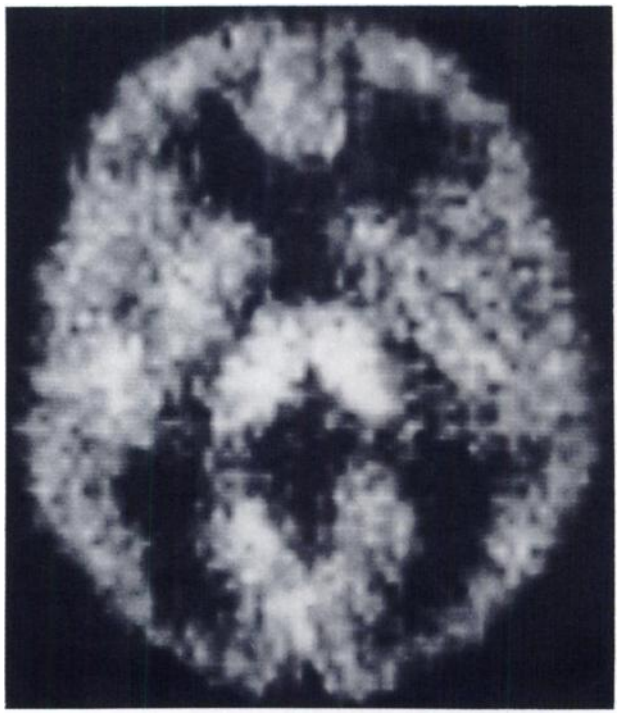

FIGURE 5
PET image of a volunteer at 3–30 min after intravenous injection of [^{11}C]DMPEA. This image was obtained at a level of 46 mm above the canthomeatal line.

0.2 ± 0.1 %dose/g. Pretreatment with clorgyline did not lower the radioactivity level significantly in the brain 60 min after injection (Fig. 3). These results indicate that [^{11}C]DMPEA is metabolized selectively by MAO-B and the radioactivity in the mouse brain 60 min after injection correlates well with MAO-B activity in the brain.

Kinetics in Humans

The accumulation of ^{11}C radioactivity in the brain was high and rapid within 4–6 min of intravenously injected [^{11}C]DMPEA and then increased gradually until the end of the experiment (Fig. 4). A high accumulation of ^{11}C radioactivity was observed in the thalamus, basal ganglia, cerebral cortex, and cerebellum (Fig. 5, Table 2). A moderate concentration of ^{11}C radioactivity was seen in the brain stem. The radioactivity in the blood decreased rapidly and was much lower than that in the brain.

DISCUSSION

The kinetics of [^{11}C]DMPEA in the mouse brain was the same as that of [^{14}C]DMPEA, which had been previously reported (*16*). Initial rapid clearance of ^{11}C radioactivity in the brain reflects the elimination of

TABLE 2
Distribution of Radioactivity in the Human Brain After Intravenous Injection of [^{11}C]DMPEA

Brain region	0–2 min	5–6 min	9–10 min	29–30 min	59–60 min*
Frontal cortex	4.2 ± 2.7	8.9 ± 2.8	9.4 ± 2.8	9.9 ± 2.7	11.7 ± 3.2
Temporal cortex	4.5 ± 2.4	8.9 ± 2.4	9.5 ± 2.3	9.6 ± 2.4	11.2 ± 1.6
Occipital cortex	4.6 ± 2.4	9.3 ± 1.9	9.8 ± 2.4	10.3 ± 2.9	10.9 ± 0.8
Basal ganglia	4.5 ± 2.5	9.0 ± 2.1	10.1 ± 2.1	11.6 ± 2.6	11.8 ± 2.7
Thalamus	4.7 ± 1.9	9.9 ± 2.7	11.1 ± 3.1	11.8 ± 3.0	12.1 ± 2.1
Cerebellum	4.2 ± 2.0	8.4 ± 2.2	8.9 ± 2.4	9.2 ± 2.6	9.8 ± 1.0
Brain stem	3.1 ± 2.1	5.1 ± 2.9	5.7 ± 3.0	6.0 ± 3.0	7.8 ± 1.5
Blood	9.5 ± 2.8	3.3 ± 0.3	2.4 ± 0.2	1.5 ± 0.1	1.1 ± 0.1

Mean uptake ± s.d. (% dose/ml × 10^{-3}) of four subjects (*two subjects)

unmetabolized [^{11}C]DMPEA and slow clearance of ^{11}C radioactivity at 15–60 min following injection reflects retention of ^{11}C-labeled dimethylamine in the brain. The dose dependent decrease of ^{11}C radioactivity at 60 min after pretreatment with various doses of l-deprenyl suggests that MAO-B activity in the animal brain and also the human brain could be estimated by external detection method using PET.

However, kinetics of ^{11}C radioactivity in the human brain following intravenous injection of [^{11}C]DMPEA indicate that most of [^{11}C]DMPEA that entered the brain was trapped. This was probably because MAO-B activity in the human brain was much higher than that in the mouse brain. Although, to the best of our knowledge, there has been no comparative study of brain MAO-B activity between mice and humans, the MAO-B activity in the human brain has been reported to be higher than that in the rat brain (*5–7,11,19–20*).

The PET images of the human subjects showed a slightly higher accumulation of ^{11}C radioactivity in the thalamus and basal ganglia, although the difference was not statistically significant. This distribution of ^{11}C radioactivity seems to be different from that of the perfusion tracers. Because it has been reported that the MAO-B activity in the autopsied human brain is higher in the thalamus and basal ganglia than in the cerebral cortex (*8,10*), the distribution of ^{11}C radioactivity following intravenous injection of [^{11}C]DMPEA might reflect the regional distribution of MAO-B activity in the human brain.

If the MAO-B activity in the human brain is considerably higher than in the mouse brain, it may be necessary to modify the tracer to be metabolized by the brain MAO-B more slowly for the purpose of detecting the alterations of MAO-B activity in the human brain.

It has been reported that a considerable reduction in deamination occurred when the deuterium substitution was in the alpha position of beta-phenylethylamine and some other trace amines (*21*). Recently, we investigated deuterium isotope effects of [^{11}C]N,N-dimethylphenylethylamine-α,α-d_2 in vivo (*22*). The study revealed that the production rate of labeled metabolite ([^{11}C]dimethylamine) in mice was reduced significantly by substitution of the α-hydrogen of [^{11}C]DMPEA with deuterium. Thus, [^{11}C]DMPEA-α,α-d_2 is a promising tracer for in vivo measurement of MAO-B activity in the human brain.

Another possible cause for the long retention of ^{11}C radioactivity in the human brain following intravenous injection of [^{11}C]DMPEA is that unmetabolized [^{11}C]DMPEA and/or labeled metabolites was eliminated much slower in the human brain than in the mouse brain. So-called nonspecific binding sites of amines (*23*) might retain [^{11}C]DMPEA and/or labeled metabolites in the human brain and there might be large interspecies differences of nonspecific binding sites of amines between mice and humans.

In conclusion, whether or not [^{11}C]DMPEA is a suitable tracer for probing MAO-B in the human brain must await further experiments to elucidate the factors responsible for the observed irreversible trapping of ^{11}C radioactivity within brain structures.

Amine metabolism is an important aspect of human brain function and MAO plays an essential role in controlling the levels of biogenic amines. We are continuing in our attempts to devise methods by which it will be possible to measure MAO activity in the human brain.

NOTES

* CGR-MeV 960, CGR, Paris, France.

† Aldrich Chemical Co., Milwaukee, WI.

‡ Megapak SIL, JASCO, Tokyo, Japan.

§ Kindly provided by Dr. J. Knoll, Department of Pharmacology, Semmelweis University of Medicine, Budapest, Hungary.

¶ May and Baker Ltd., Dagenham, UK.

ACKNOWLEDGMENTS

This work was supported by special coordination funds for promoting science and technology in Japan.

The authors are grateful to Dr. Y. Yoshida and T. Irie for their useful advice, K. Tamate for technical assistance in the preparation of [^{11}C]DMPEA, and K. Yoshida and S. Himi for their technical assistance in the PET study.

REFERENCES

1. Murphy DL, Garrick NA, Aulakh CS, et al. New contribution from basic science to understanding the effects of monoamine oxidase inhibiting antidepressants. *J Clin Psychiat* 1984; 47:37–43.
2. Johnston JP: Some observations upon a new inhibitor of monoamine oxidase in brain tissue. *Biochem Pharmacol* 1968; 17:1285–1297.
3. Knoll J, Magyar K. Some puzzling pharmacological effects of monoamine oxidase inhibitors. *Adv Biochem Psychopharmacol* 1972; 5:393–408.
4. Houslay MD, Tipton KF. Multiple forms of monoamine oxidase: Fact and artefact. *Life Sci* 1976; 19:467–478.
5. Shih JC. Monoamine oxidase in aging human brain. In: Singer TP, Von Korff RW, Murphy DL, eds. Monoamine oxidase; Structure, function, and altered functions. New York: Academic Press, 1979:413–421.
6. Oreland L, Fowler CJ. The activity of human brain and thrombocyte monoamine oxidase (MAO) in relation to various psychiatric disorders. II) The nature of the changed MAO activity. In: Singer TP, Von Korff RW, Murphy DL, eds. Monoamine oxidase; Structure, function, and altered functions. New York: Academic Press, 1979:389–396.
7. Fowler CJ, Wiberg A, Oreland L, et al. The effect of age on the activity and molecular properties of human

brain monoamine oxidase. *J Neural Transm* 1980; 49:1–20.
8. Eckert B, Gottfries CG, Von Knorring L, et al. Brain and platelet monoamine oxidase in mental disorders. 1. Schizophrenics and cycloid psychotics. *Prog Neuro-Psychopharmacol* 1980; 4:57–68.
9. Adolfsson R, Gottfries CG, Oreland L, et al. Increased activity of brain and platelet monoamine oxidase in dementia of Alzheimer type. *Life Sci* 1980; 27:1029–1034.
10. Oreland L, Arai Y, Stenström A, et al. Monoamine oxidase activity and localisation in the brain and the activity in relation to psychiatric disorders. *Mod Probl Pharmacopsychiat* 1983; 19:246–254.
11. Oreland L, Arai Y, Stenström A. Age, neuro-psychiatric diseases and brain monoamine oxidase. In: Tipton KF, Benedetti PD, Benedetti MS, eds. Monoamine oxidase and disease. London: Academic Press, 1984:291–297.
12. MacGregor RR, Halldin C, Fowler JS, et al. Selective, irreversible in vivo binding of [^{11}C]clorgyline and [^{11}C]-l-deprenyl in mice: potential for measurement of functional monoamine oxidase activity in brain imaging positron emission tomography. *Biochem Pharmacol* 1985; 34:3207–3210.
13. Ishiwata K, Ido T, Yanai K, et al. Biodistribution of a positron-emitting suicide inactivator of monoamine oxidase, carbon-11 pargyline, in mice and a rabbit. *J Nucl Med* 1985; 26:630–636.
14. Inoue O. A new metabolically trapped agent by brain monoamine oxidase: N-methyl labelled [^{14}C] N-methyl-phenylethylamine (^{14}C-MPEA). *Eur J Nucl Med* 1983; 8:385–388.
15. Inoue O, Tominaga T, Yamasaki T, et al. A new method for in vivo measurement of brain monoamine oxidase activity. *Prog Neuro-Psychopharmacol Biol Psychiat* 1984; 8:385–395.
16. Inoue O, Tominaga T, Yamasaki T, et al. Radioactive N,N-dimethylphenylethylamine: A selective radiotracer for in vivo measurement of monoamine oxidase-B activity in the brain. *J Neurochem* 1985; 44:210–216.
17. Hall HK. Correlation of the base strengths of amines. *J Am Chem Soc* 1957; 79:5441–5444.
18. Takami K, Ueda K, Okajima K, et al. Performance of whole-body, multislice positron computed tomograph—Positologica II—. *IEEE Trans Nucl Sci* 1983; 30:734–738.
19. Student AK, Edwards DJ. Subcellular localization of types A and B monoamine oxidase in rat brain. *Biochem Pharmacol* 1977; 26:2337–2342.
20. Benedetti MS, Kean PE. Differential changes in monoamine oxidase A and B activity in the aging rat brain. *J Neurochem* 1980; 35:1026–1032.
21. Yu PH, Barclay S, Davis B, et al. Deuterium isotope effects on the enzymatic oxidative deamination of trace amines. *Biochem Pharmacol* 1981; 30:3089–3094.
22. Hashimoto K, Inoue O, Suzuki K, et al. Deuterium isotope effect of [^{11}C]N,N-dimethylphenethyl-amine-α,α-d_2; Reduction in metabolic trapping rate in brain. *Int J Nucl Med Biol* 1986; 13:79–80.
23. Winchell HS, Baldwin RM, Lin TH. Development of I-123-labeled amines for brain studies: Localization of I-123 indophenylalkyl amines in rat brain. *J Nucl Med* 1980; 21:940–946.