Biochemical Pharmacology, Vol. 36, No. 21, pp. 3671–3675, 1987.
Printed in Great Britain.

0006-2952/87 $3.00 + 0.00

[^{13}N]-β-PHENETHYLAMINE ([^{13}N]PEA): A PROTOTYPE TRACER FOR MEASUREMENT OF MAO-B ACTIVITY IN HEART

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(*Received* 3 *November* 1986; *accepted* 24 *March* 1987)

Abstract—[^{13}N]β-Phenethylamine ([^{13}N]PEA) was evaluated as a radio tracer for the measurement of mouse heart monoamine oxidase (MAO) activity *in vivo*. After intravenous administration, [^{13}N]PEA was deaminated by MAO-B. $^{13}NH_3$ formed thereby was taken up by amino acids and trapped in the heart. The relation between the radioactivity trapped in the heart and the enzyme activity was examined. The radioactivity in the heart 15 min after administration was reduced in a dose-dependent manner by pretreatment with a specific MAO-B inhibitor, *l*-deprenyl, but not with a specific MAO-A inhibitor, clorgyline. A linear correlation existed between the heart radioactivity level and the heart MAO-B activity (0–45%). [^{13}N]1,1-d_2-2-Phenethylamine (C_6H_5-CH_2-CD_2-$^{13}NH_2$, [^{13}N]d_2PEA), a modified tracer with less reactivity towards the enzyme, was tested similarly. This tracer possessed a higher sensitivity than [^{13}N]PEA, and a wider range (0–85%) of MAO-B activity correlated linearly with the trapped radioactivity. These results indicate that [^{13}N]PEA derivatives ([^{13}N]PEA and [^{13}N]d_2PEA) can be useful radiotracers for noninvasive measurements of MAO-B activity in the human heart.

Monoamine oxidase (EC 1.4.3.4, amine:O_2 oxidoreductase, MAO) plays a major role in the metabolism of both endogenous and exogenous amines. MAO has been subdivided into MAO-A and MAO-B according to substrate and inhibitor specificities. Several studies have been carried out to clarify the function of each form of MAO under physiological as well as under pathological conditions. The positron tracer technique, which uses tracers labeled with positron-emitting nuclides (^{11}C, ^{13}N, ^{18}F, etc.), and positron emission tomography (PET) are useful methods for the noninvasive measurement of the rates of transport, metabolism and excretion of various substances in humans and animals *in vivo*. So is [^{18}F]deoxyglucose which is widely used to study cerebral metabolic rates of glucose [1].

In several studies, positron tracer techniques have been applied in the estimation of MAO activity *in vivo* [2–4]. Recently, we developed [^{11}C]*N,N*-dimethylphenylethylamine as a tracer for the measurement of brain MAO-B activity, utilizing the principle of metabolic trapping [5].

Previously, we had reported [6] the synthesis of several [^{13}N]amines as well as their distribution and metabolism after injection in mice. After administration of these [^{13}N]amines, the radioactivity is metabolically trapped in the cardiac muscle. The amines are metabolized by MAO into $^{13}NH_3$, which is then trapped as part of labeled amino acids. The unmetabolized amines are excreted from the organ. Since the radioactivity is trapped in proportion to the amount of $^{13}NH_3$, the heart radioactivity may correlate with the activity of MAO. Based on this assumption, we are designing tracers for the estimation of heart MAO activity *in vivo*.

In this study, we evaluated [^{13}N]β-phenethylamine ([^{13}N]PEA) as a prototype tracer and found that changes in MAO-B activity in mice hearts were selectively detected and that the sensitivity of the tracer towards the enzyme activity was changed by chemical modification.

MATERIALS AND METHODS

Materials. Clorgyline hydrochloride (CLO) and *l*-deprenyl hydrochloride (DPL) were provided by May & Baker Ltd., Dagenham, U.K. and Dr. J. Knoll, Department of Pharmacology, Semmelweis University of Medicine, Budapest, Hungary, respectively. Other chemicals and solvents were of the highest grade commercially available.

Preparation of [^{13}N]PEA and [^{13}N]d_2PEA. [^{13}N]PEA and [^{13}N]d_2PEA were synthesized according to a method we published previously [7]. Briefly, [^{13}N]phenylacetamide was synthesized from phenylacetyl chloride and [^{13}N]ammonia; the intermediate amide was reduced by $LiAlH_4$ to yield [^{13}N]PEA and by $LiAlD_4$ to yield [^{13}N]d_2PEA. The radiochemical purities and the specific activities of the products were over 90% and about 100 Ci/mmol respectively.

Time course of heart and blood radioactivity levels after i.v. administration of [^{13}N]PEA. Aqueous solutions of [^{13}N]PEA hydrochloride (prepared in a non-carrier-added state, 0.2 ml, about 30 μCi/mouse,

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1.2 μg/kg) were injected into tail veins of male C3H mice (10–12 weeks, about 30 g). After given time intervals, the animals were killed, blood and hearts were removed and weighed, and their radioactivity levels were determined in a well-scintillation counter. The results are expressed in terms of percent dose per g heart or blood respectively. In the study of the effect of MAO inhibition, pargyline hydrochloride (PGL, 100 mg/kg, i.p.) was administered 1 hr before the injection of the tracer.

Effect of specific MAO inhibitors on the amount of radioactivity trapped in the heart after administration of [^{13}N]*PEA or* [^{13}N]d_2*PEA.* CLO (MAO-A inhibitor, 0.1 to 10 mg/kg) or DPL (MAO-B inhibitor, 0.01 to 10 mg/kg) was injected into the tail veins of male C3H mice. One hour after this pretreatment, [^{13}N]PEA or [^{13}N]d_2PEA was injected intravenously and 15 min later the mice were killed; hearts and blood were removed and weighed, and their radioactivity levels were determined. The results are expressed in terms of percent dose per g heart or blood respectively.

Analysis of the radioactive metabolites after i.v. administration of [^{13}N]*PEA.* [^{13}N]PEA was injected into tail veins of control mice or mice pretreated with either CLO (10 mg/kg, i.v.) or DPL (10 mg/kg, i.v.). Three or 15 minutes later, the mice were killed, and the hearts were removed and homogenized in 2 ml of physiological saline. The homogenates were basified with NaOH and extracted with water-saturated ethyl acetate. The extraction efficiency was determined by comparing the radioactivity in the extract and that in the homogenate. The extract of the homogenate from DPL-pretreated mice was analyzed using thin-layer chromatography [TLC, silica gel, chloroform–methanol (2:1)].

In vitro *assay of MAO activity in heart and liver.* MAO activity in mice was determined radiometrically *in vitro* as described previously [8]. The tissue homogenate was incubated with a final concentration of 0.01 mM [^{14}C]PEA (as a substrate for MAO-B) or 0.01 mM [^{14}C]5-HT (as a substrate for MAO-A) at 37° and pH 7.4. The incubation period was 10 min with 5-HT and 5 min and PEA. The amount of product formed was found to be proportional to the incubation time and the amount of enzyme preparation used. For the determination of *in vivo* inhibition of MAO activity, C3H mice were pretreated with various doses of either CLO or DPL 1 hr before they were killed, and the enzyme activity was measured as described above.

RESULTS

Figure 1 shows the time courses of the radioactivity in the heart after the administration of [^{13}N]PEA in control and pargyline-pretreated mice. MAO inhibition hindered the retention of the radioactivity in the heart, though there was little change in the blood radioactivity (see Ref. 6 for the radioactivity in organs other than the heart). The effects of specific MAO inhibitors on the radioactivity trapped in the heart after the injection of [^{13}N]PEA are shown in Fig. 2. The radioactivity levels were decreased markedly and in a dose-dependent manner by the pretreatment with the specific MAO-B inhibitor DPL, whereas the specific MAO-A inhibitor CLO had no significant effect up to a dose of 10 mg/kg.

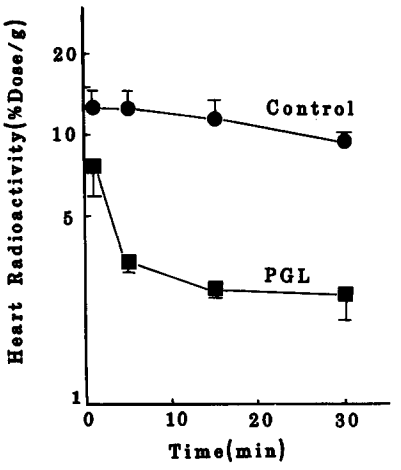

Fig. 1. Time course of heart radioactivity after intravenous administration of [^{13}N]PEA in control mice and PGL-pretreated mice. Radioactivities are expressed as percent dose per gram organ. Values are expressed as averages ±1 SD of three mice for each point.

The extraction efficiency of radioactive material from the heart homogenate after the administration of [^{13}N]PEA was low unless the animal had been pretreated with DPL (Table 1). Over 86% of the radioactivity in extracts from the hearts of DPL-pretreated mice was that of unmetabolized [^{13}N]PEA.

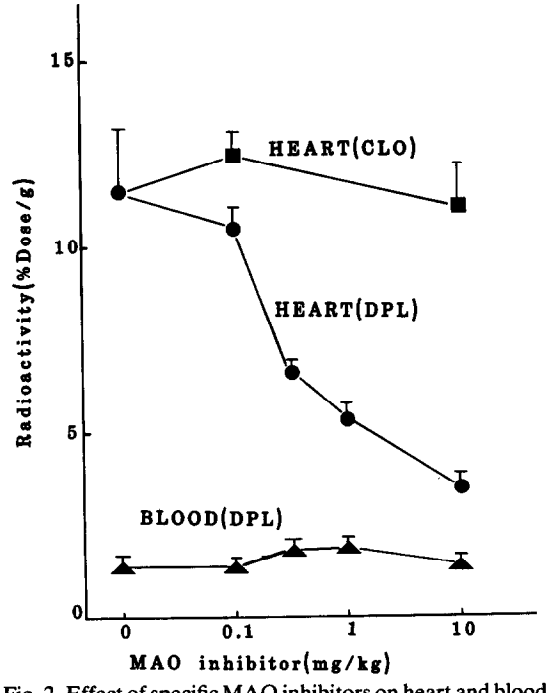

Fig. 2. Effect of specific MAO inhibitors on heart and blood radioactivities 15 min after administration of [^{13}N]PEA. Radioactivities are expressed as percent dose per gram organ. Values are expressed as averages ±1 SD of three mice for each point.

Table 1. Extractable metabolites in the heart after i.v. administration of [^{13}N]PEA

Pretreatment	Time after i.v. (min)	Extraction efficiency* (%)
None	3	5
	15	4
DPL	3	79
	15	60
CLO	3	3
	15	4

* The homogenate of a heart was basified and extracted with water-saturated ethyl acetate as described in the text; each value given is the mean of two mice.

MAO-A and MAO-B activities remaining in the hearts and the livers of mice pretreated with specific MAO inhibitors are summarized in Table 2. CLO as well as DPL keeps its MAO subtype specificity also *in vivo*.

Plots of the radioactivity trapped in the heart (Fig. 2) against the activity of two forms of MAO remaining after the pretreatment with CLO or DPL (Table 2) are shown in Fig. 3. The radioactivity was proportional to the percentage of remaining MAO-B activity in a range between 0 and about 45%, but it did not correlate with the MAO-A activity (CLO pretreatment).

The effect of DPL on the heart radioactivity after the administration of [^{13}N]d_2PEA was compared with that after the administration of [^{13}N]PRA (Fig. 4). Substitution of the α-position with deuteriums markedly increased the sensitivity of the tracer to DPL. An appreciable decrease in the radioactivity was observed only at the DPL doses over 0.3 mg/kg

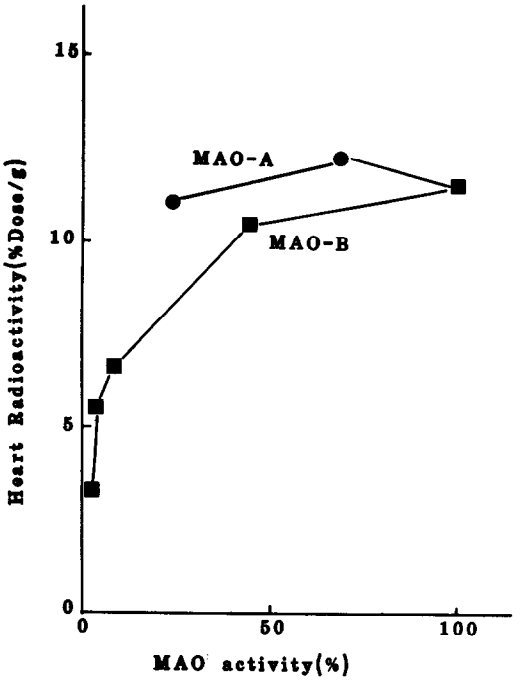

Fig. 3. Relation between MAO activity and heart radioactivity after administration of [^{13}N]PEA. Radioactivities are expressed as percent dose per gram organ. Values are expressed as averages of three mice for each point.

Table 2. Remaining NAO activity in mice hearts and livers after pretreatment with various doses of CLO and DPL respectively

MAO inhibitor (mg/kg)	Percent of initial enzyme remaining			
	Heart		Liver	
	MAO-A	MAO-B	MAO-A	MAO-B
CLO				
0.1	68.1	116.8	110.3	126.1
10	25.3	87.1	32.0	90.0
DPL				
0.01	98.6	84.5	74.0	109.3
0.1	110.8	43.8	91.1	90.0
0.33	68.9	7.6	66.5	82.2
1.0	37.9	3.8	45.5	35.7
10	45.4	3.5	40.0	9.0

Various doses of either CLO or DPL were injected intraveneously into mice 1 hr before decapitation. Remaining activities were assayed as described in the text and expressed as percentages of the control activity. Each value is the mean of three mice, MAO-A activities of the heart and the liver were 0.08 and 0.19 nmol product formed per mg of protein per min respectively. MAO-B activities of the heart and the liver were 1.1 and 3.8 nmol product formed per mg protein per min respectively.

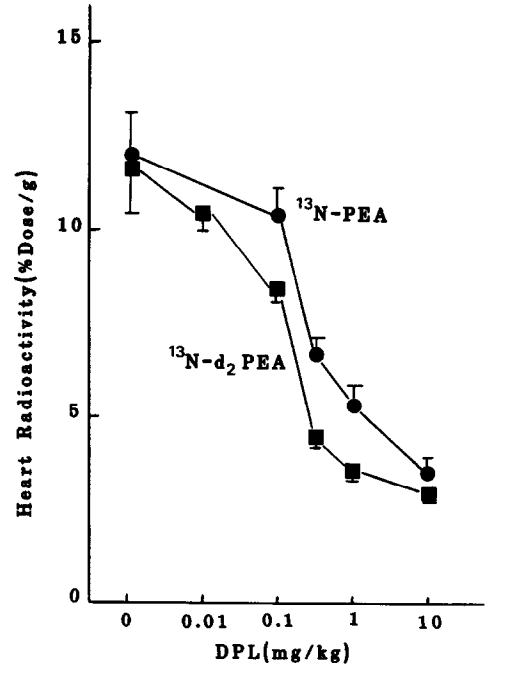

Fig. 4. Effect of DPL on heart radioactivity 15 min after administration of either [^{13}N]PEA or [^{13}N]d_2PEA. Radioactivities are expressed as percent dose per gram organ. Values are expressed as averages ±1 SD of three mice for each point.

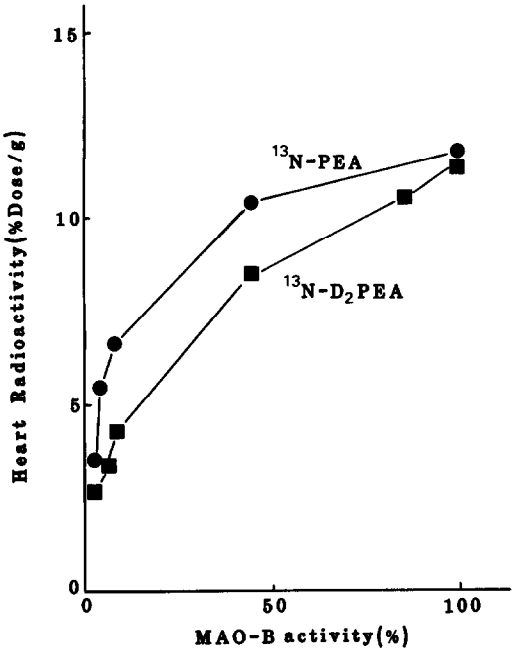

Fig. 5. Relation between MAO-B activity and heart radioactivity after administration of either [^{13}N]PEA or [^{13}N]d$_2$PEA. Radioactivities are expressed as percent dose per gram organ. Values are expressed as averages of three mice for each point.

in the case of [^{13}N]PEA, whereas in the case of [^{13}N]d$_2$PEA, it was observed at doses over 0.1 mg/kg. [^{13}]d$_2$PEA afforded a lower level of trapped radioactivity in the hearts of mice that had been pretreated with equal dose of DPL. Furthermore, a wider range of the MAO-B activity (0–85%) showed a linear correlation with the trapped radioactivity (Fig. 5).

DISCUSSION

Recent advances in positron-tracer techniques have opened up the possibility of using various organic compounds as tracers to study various physiological functions. In the field of cardiac nuclear medicine, ^{11}C-labeled tracers have been used to study fatty acid metabolism [9] and β-receptor functions [10] in the heart. However, there have been few studies using this tracer technique to clarify degradation processes of the transmitting amines, an important aspect in the regulatory system of a heart. Therefore, we have tried to develop a tracer for the estimation of myocardial MAO activity.

Three different methods using the tracer technique for the estimation of MAO activity *in vivo* have been reported. The first one by Gallagher *et al.* [2] is the radiorespirometric method, e.g. the measurement of expired $^{11}CO_2$ after injection of α[^{11}C]-*n*-octylamine. However, the rate-determining step in the $^{11}CO_2$ excretion has been reported to occur after the deamination. The second one uses ^{11}C-labeled MAO inhibitors, [^{11}C]deprenyl, [^{11}C]clorgyline, and [^{11}C]pargyline, as tracers [3, 4]. This method has been reported to be useful to assay MAO activity *in vivo*, though the correlation between tracer uptake and MAO activity in organs has not yet been established.

The third method uses a labeled substrate as a tracer, and the radioactivity of a metabolite trapped in the target organ is measured. In our previous report [5], we evaluated [^{11}C]*N,N*-dimethylphenylethylamine ([^{11}C]DMPEA) as a substrate tracer to measure the brain MAO-B activity *in vivo*. After intravenous administration, this tracer rapidly enters the brain and is selectively deaminated by MAO-B into [^{11}C]dimethylamine which is then trapped by the blood–brain barrier (BBB). The unmetabolized tracer is rapidly eliminated from the brain so that the radioactivity trapped in the brain is in proportion to the brain MAO-B activity. The same principle seems to be applicable in the estimation of MAO activity in other organs. However, [^{11}C]DMPEA itself cannot be used for this purpose, because [^{11}C]dimethylamine is trapped only by the BBB in the brain and is eliminated rapidly from other organs such as the heart.

After intravenous injection, [^{13}N]amines liberate $^{13}NH_3$ upon MAO oxidation. This $^{13}NH_3$ is converted into labeled amino acids (mainly [^{13}N]glutamine) and retained in the organ according to metabolic turnover (Ref. 6; for the metabolic fate of $^{13}NH_3$, see Ref. 11). In the mouse heart, the labeled amino acids are trapped for at least 30 min after administration, while unmetabolized amines are rapidly excreted from the organ (Ref. 6, Fig. 1). Since $^{13}NH_3$-fixing enzymes (mainly glutamine synthetase) exist in large excess to the $^{13}NH_3$ liberated in the heart and the conversion proceeds very rapidly [11], the rate-determining step of the trapping of the radioactivity should be the MAO oxidation. After elimination of the unmetabolized tracer, all the radioactivity in the heart corresponds to that of the labeled amino acids whose amount correlates with the MAO activity. Therefore, the myocardial MAO activity can be assayed via the radioactivity in the heart.

We selected [^{13}N]PEA as a prototype substrate tracer for the following reasons. (1) PEA is a specific substrate for MAO-B, and the effects of chemical modification such as β-hydroxylation or α-deuteration on the properties have been well studied. (2) [^{13}N]PEA can be rapidly synthesized in a good yield according to the method described in our previous paper [7]. (3) [^{13}N]PEA shows high heart uptake and is therefore suitable for external measurement of the heart radioactivity. We measured the heart radioactivity 15 min after the injection of the tracer, because by that time the metabolism as well as the elimination of unmetabolized tracer were found to be completed (Fig. 1, Table 1).

As shown in Fig. 2, the heart radioactivity 15 min after intraveneous injection of [^{13}N]PEA was reduced in a dose-dependent manner by DPL pretreatment but not by CLO pretreatment. This reduction is accompanied by the hindrance of metabolism of the tracer (Table 1). Although the pretreatment with 0.33 mg/kg of DPL had only a slight effect on the MAO-B activity in the liver (Table 2), the main amine-metabolizing organ, it significantly

reduced the radioactivity trapped in the heart. The radioactivity in the blood was low (about 2% dose/g) and remained unchanged by the pretreatment. These facts show that the determining factor for heart radioactivity was the MAO-B activity in the heart and not the metabolism of the tracer in other organs or the unchanged or metabolized tracer in the blood. For detailed studies on human myocardial MAO activity using PET, however, the amount of the unchanged tracer as well as that of the metabolites in the blood would have to be measured in order to achieve accurate quantitation of the enzyme activity.

As shown in Fig. 3, there is a good correlation between the radioactivity trapped in the heart and the activity of heart MAO-B, but not that of MAO-A. This fact clearly indicates that [^{13}N]PEA can be used as a prototype tracer for the measurement of human heart MAO-B activity by external detection (PET). However, we observed direct linearity only when the MAO-A activity was in a range of 0–45% of the control activity. Accordingly, a small change in the enzyme activity cannot be detected with this tracer.

Model considerations of metabolic trapping of a labeled tracer [12] revealed that the measurable range (range of an enzyme activity showing linear correlation with the trapped radioactivity) depends primarily on the rate of metabolism of a substrate tracer. The relationship between the upper limit of the measurable range and the rate of the metabolism is expressed in the following equation:

$$UL = C \times K_{el}/K_m$$

where UL is the ratio of the upper limit to the total enzyme activity, C is a constant which depends on the detector such as a positron camera, K_{el} is the rate constant of the elimination of the tracer from the organ, and K_m is the rate constant of the metabolism of the tracer. From this equation, the following are clear. If a tracer is metabolized very easily, an appreciable decrease in the trapped radioactivity appears only when the enzyme activity is reduced severely, whereas a tracer that is metabolized slowly is suitable for detecting a small decrease in activity. Therefore, in order to upgrade the measurable range of the MAO-B activity, a tracer with a lower reactivity than that of [^{13}N]PEA but an unchanged specificity towards the enzyme should be used.

According to Yu *et al.* [13], deuterium substitution in the α-position of PEA reduces its reactivity (V_{max}) to half but does not reduce the specificity towards the subtypes of MAO. Taking advantage of this fact, we adopted [^{13}N]d_2PEA as a modified tracer. As expected, a smaller decrease in the enzyme activity could be detected using [^{13}N]d_2PEA and, consequently, the MAO-B range that shows a linear correlation with the heart radioactivity was extended (Figs. 4 and 5). Thus, one advantage of this method is that the property of the tracer can be adjusted to the respective needs. In a clinical application, the tracer should be modified so as to match the human heart MAO activity.

In conclusion, [^{13}N]PEA derivatives ([^{13}N]PEA and [^{13}N]d_2PEA) were found to be useful radiotracers for the measurement of heart MAO-B activity by external detection. It was also shown that the property of the tracer can be adjusted by chemical modification.

Acknowledgement—The authors are grateful to Mr. K. Tamate for the isotope production.

REFERENCES

1. M. E. Phelps, S. C. Huang, E. J. Hoffmann, C. Slein, L. Sokoloff and D. E. Kuhl, *Ann. Neurol.* **6**, 371 (1979).
2. B. M. Gallagher, J. S. Fowler, R. R. MacGregor and A. P. Wolf, *Biochem. Pharmac.* **26**, 1917 (1977).
3. R. R. MacGregor, C. Halldin, J. S. Fowler, A. P. Wolf, C. D. Arnett, B. Langstrom and D. Alexoff, *Biochem. Pharmac.* **34**, 3207 (1985).
4. K. Ishiwata, T. Ido, K. Yanai, K. Kawashima, Y. Miura, M. Monma, S. Watanuki, T. Takahashi and R. Iwata, *J. nucl. Med.* **26**, 630 (1985).
5. O. Inoue, T. Tominaga, T. Yamasaki and H. Kinemuchi, *J. Neurochem.* **44**, 210 (1985).
6. T. Tominaga, O. Inoue, K. Suzuki, T. Yamasaki and M. Hirobe, *Int. J. nucl. Med. Biol.*, in press.
7. T. Tominaga, O. Inoue, K. Suzuki, T. Yamasaki and M. Hirobe, *Appl. Radiat. Isot.* **37**, 1209 (1986).
8. C. J. Fowler and L. Oreland, *Biochem. Pharmac.* **29**, 2225 (1980).
9. E. Livni, D. R. Elmaleh, S. Levy, G. L. Brownell and W. H. Strauss, *J. nucl. Med.* **23**, 169 (1982).
10. A. Syrota, D. Dormont, J. Berger, M. Maziere, C. Prenant, J. Sastre, J. M. Davy, M. C. Aumant, J. Motte and R. Gourgon, *J. nucl. Med.* **24**, p 20 (1983).
11. H. R. Schelbert, M. E. Phelps, S. Huang, N. S. MacDonald, H. Hansen, C. Selin and D. E. Kuhl, *Circulation* **63**, 1259 (1981).
12. O. Inoue, T. Tominaga, N. Fukuda, K. Suzuki and T. Yamasaki, *Jap. J. nucl. Med.* **21**, 671 (1984).
13. P. H. Yu, S. Barclay, B. David and A. A. Boulton, *Biochem. Pharmac.* **30**, 3089 (1981).