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Ionic interaction of [^{11}C]-*N*,α-dimethylbenzylamine (DMBA) in rodent brain

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The [S] enantiomer of [^{11}C]-*N*,α-dimethylbenzylamine (DMBA) was synthesized by N-methylation of [S]-α-methylbenzylamine, and its biodistribution in mice was measured. [^{11}C]-[S]-DMBA was rapidly distributed into the brain, heart and lungs, and considerable long-term retention in the brain was observed. The radioactive metabolites in the plasma were analyzed by liquid chromatography. Kinetic analysis using unmetabolized [^{11}C]DMBA in the plasma as the input function was performed employing a simplified two-compartment model. The estimated distribution volumes (DV) of [^{11}C]DMBA in the brain and heart were 6.05 and 3.95, respectively. The right striatum of the rat brain was lesioned with ibotenic acid 2 weeks before the tracer experiment. Both *in vitro* and *in vivo* autoragiographic studies were performed, and revealed significant reduction of the radioactivity levels in the lesioned striatum. On the other hand, the regional cerebral blood flow, as measured by [^{14}C]iodoantipyrine, was not significantly altered in the lesioned striatum. These results indicate that the ionic binding component for DMBA exists mainly in neural cells rather than in glial cells. [^{11}C]DMBA might be a useful radiotracer for detection of neural cell loss in the brain.

Key words: dimethylbenzylamine (DMBA), brain, neuron, ibotenic acid

INTRODUCTION

AMPHETAMINE and methamphetamine have been reported to exert a wide variety of pharmacological actions.[1] These amines rapidly enter the brain and accumulate in neural tissue.[2] The long-term retention observed in the brain indicates that binding components for these amines may be present in the CNS. In a previously reported study, we synthesized[3] and compared the kinetic properties of [^{11}C]labeled methamphetamine (MAMP) and β,β-difluoromethamphetamine (DFMAMP) in the pig brain.[4] While more than 99% of MAMP is protonated at physiological pH (7.4) due to the high pKa[10,11] of its amino group, only 50% of DFMAMP, whose pKa is lower (7.2), is protonated at this pH. Furthermore, while [^{11}C]DFMAMP is rapidly distributed into the pig brain and its elimination rate from the brain is also rapid, long-term retention of [^{11}C]MAMP in the brain is observed. These PET data indicate that an ionic interaction rather than hydrophobic interaction of these basic amines within the brain tissue is an important intention for the binding of these amines in the brain. Significant decreases in [^{123}I]iodoamphetamine in primary brain tumors were reported, regardless of increase or decrease in the blood flow in the tumors,[5] which suggested that the binding components for the amines might exist in neural cells rather than in glial cells. In this study, we synthesized [^{11}C]-*N*,α-dimethylbenzylamine, a methamphetamine analogue, and evaluated its binding properties in the brains of small rodents. In order to determine whether the binding components exist in neural cells, lesions were induced with ibotenic acid in the rat striatum, and both *in vitro* and *in vivo* autoradiograhic studies were performed.

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MATERIALS AND METHODS

Animals

Male ddY mice (8 weeks old) and Wistar rats (8 weeks old) were purchased from SLC (Hamamatsu, Japan) and housed in cages maintained at 23°C and a 12-hr light-dark cycle. All the animals were given free access to food and water. The studies were performed with the permission of the Institutional Animal Care and Use Committee, School of Allied Health Sciences, Osaka University.

Chemicals

[S]-α-methylbenzylamine was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). [^{14}C]iodoantipyrine, specific activity 1875 MBq/mmol, was obtained from New England Nuclear (Boston, MA, USA). The other chemicals used were of the highest grade commercially available.

Method

Synthesis of the [S] enantiomer of [^{11}C]-N,α-dimethylbenzylamine (DMBA)

Both enantiomers of [^{11}C]DMBA were synthesized by *N*-methylation of α-methylbenzylamine. [^{11}C]methyliodide was trapped in dimethyformamide (DMF) containing sodium hydride (NaH) and α-methyl benzylamine (1 mg), and reaction was allowed to occur for 5 min at 70°C. [^{11}C]DMBA was purified by liquid chromatography on Megapak SIL C18 (JASCO, Tokyo, Japan) eluted with acetonitrile: 5 mM phosphoric acid (30%, 70%). After evaporation of the eluant to dryness, the residue was dissolved in saline and filtered by passage over a 0.22 μm of membrane filter. Specific radioactivity was more than 7.4 GBq/μmol.

The time-course of changes in the radioactivity levels following injection of [^{11}C]DMBA

Mice were intravenously injected with 3.7 MBq of [^{11}C]DMBA, and sacrificed by decapitation under light ether anesthesia, 1, 5, 10, 20 and 30 min after the tracer injection. After sacrifice, the blood was collected and centrifuged for 5 min (1500 G). The brain, heart and lungs were quickly removed, and the brain dissected into cerebral cortex, striatum and cerebellum, and weighed. The radioactivity level in each sample was measured with a well scintillation counter, and decay corrections were made. The values were expressed as a percentage of the injected dose per gram of wet tissue (%Dose/g).

Metabolite analysis in plasma samples following injection of [^{11}C]DMBA

After adding an equal volume of acetonitrile (CH_3CN) solution to the plasma samples (0.7 m*l*) obtained above, the precipitate was removed by centrifugation (1500 G, 1 min, 4°C). The supernatant was analyzed by liquid chromatography with 0.01 M phosphoric acid/ CH_3CN as the eluant (flow rate: 2.5 m*l*/min).

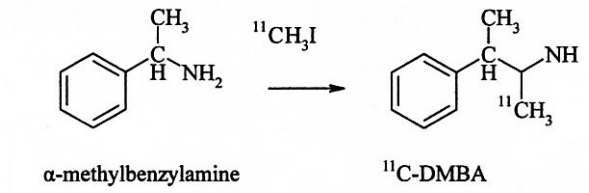


Fig. 1 Synthesis of [^{11}C]*N*,α-dimethylbenzylamine with [^{11}C]methyliodide.

Labeled metabolites and unmetabolized [^{11}C]DMBA were quantified using an UV absorbance detector (254 nm) and a sensitive positron detector.[6]

Kinetic analysis of [^{11}C]DMBA binding in the mouse brain, heart and lungs

The time course of changes in the radioactivity levels of unmetabolized [^{11}C]DMBA in the plasma was determined by metabolite correction as described above, and used as the input function. The simplified two-compartment model was employed for estimation of the distribution volume ($DV = k_1/k_2$) of [^{11}C]DMBA.

Lesioning of the right striatum of the rat brain with ibotenic acid

Rats were anesthetized with pentobarbital (50 mg/kg) and placed in a stereotaxic apparatus. A stainless steel injection cannula (33 gauge) was inserted into the striatum, identified according to the Atlas of Paxinos and Watson: 0.2 mm anterior to the bregma, 3.2 mm lateral to the midline, and 4.5 and 6.5 mm below the cortical surface. Ibotenic acid (8 μg/μ*l*) was infused twice at the rate of 1 μ*l*/min over 2 min. Two weeks after the infusion, the rats were used for both *in vitro* and *in vivo* autoradiographic experiments.

In vivo *autoradiography*

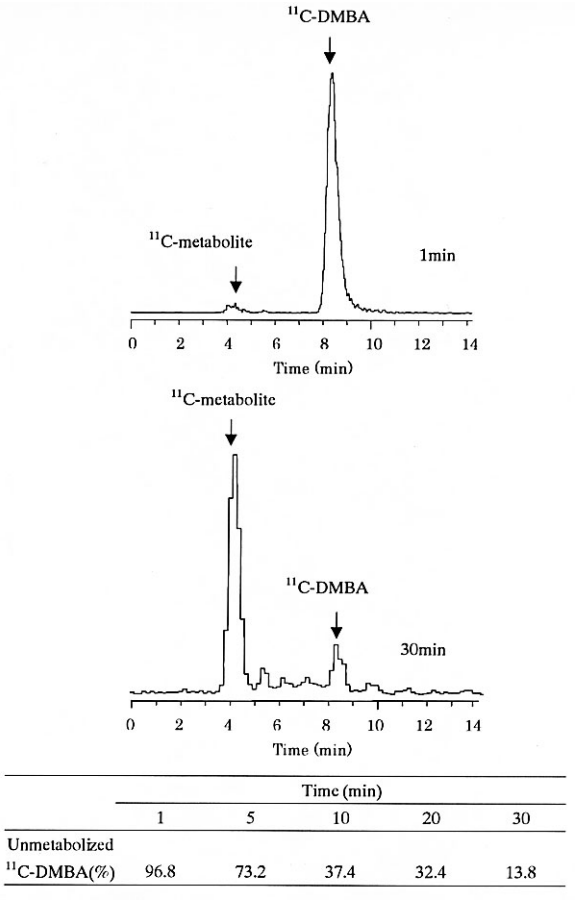
Rats were intravenously injected with 185 MBq of [^{11}C]DMBA, and decapitated 5 min post injection of the tracer. The brains were quickly removed, and brain slices (2 mm in thickness) were prepared immediately. For measurement of the regional blood flow, rats were intravenously injected with [^{14}C]iodoantipyrine (370 kBq/rat; New England Nuclear, Boston, MA, USA) over 1 min, and immediately decapitated.[7] The brains were quickly removed and frozen, and coronal sections (20 μm) were prepared in a cryostat at −20°C.

The sections were exposed to an imaging plate (Fuji Photo Film), and autoradiograms were quantified using photo-stimulated luminescence (PSL) values with a Bio-Imaging Analyzer (Bas 3000, Fuji Photo Film). The radioactivity levels in the regions of interest were determined as PSL/area (mm^2), and values were expressed as the relative ratios of the radioactivity levels in the right

Table 1 Tissue distribution of [^{11}C]DMBA in mice

Time (min)	Brain	Heart	Lung	Plasma
1	3.66 ± 1.77	3.38 ± 1.76	4.53 ± 2.48	0.82 ± 0.39
5	4.10 ± 0.32	2.42 ± 0.16	4.27 ± 0.36	0.82 ± 0.07
10	3.05 ± 0.39	2.06 ± 0.14	3.99 ± 0.39	1.08 ± 0.16
20	2.39 ± 0.28	1.64 ± 0.08	2.98 ± 0.28	0.94 ± 0.05
30	1.59 ± 0.26	1.50 ± 0.19	2.27 ± 0.54	1.03 ± 0.11

Values are mean ± 1 s.d. of four mice in each group



	Time (min)				
	1	5	10	20	30
Unmetabolized ^{11}C-DMBA(%)	96.8	73.2	37.4	32.4	13.8

Fig. 2 Radiochromatograms of labeled metabolite in plasma specimens 1 min and 30 min after the injection of [^{11}C]DMBA into mice. The blood samples were collected from four mice in each group, and centrifuged for 5 min. HPLC analysis with a highly sensitive radiodetector was performed by the method described in the text.

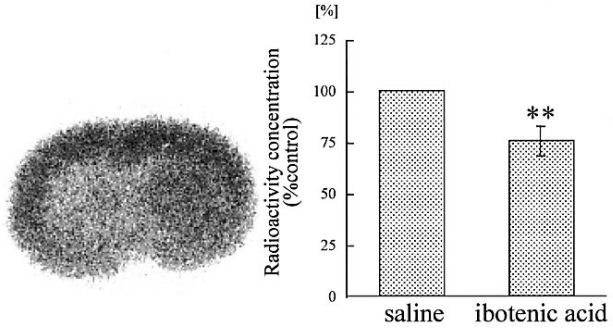


Fig. 3 *In vivo* autoradiogram of [^{11}C]DMBA in the rat brain. Rats were pretreated with ibotenic acid 2 weeks prior to the experiment, and decapitated 5 min post injection. The brains were quickly removed, and brain slices (2 mm) were prepared and treated with IP. Values are expressed as the relative radioactivity levels in the right striatum and left striatum (n = 4). *p < 0.01, compared to control side.

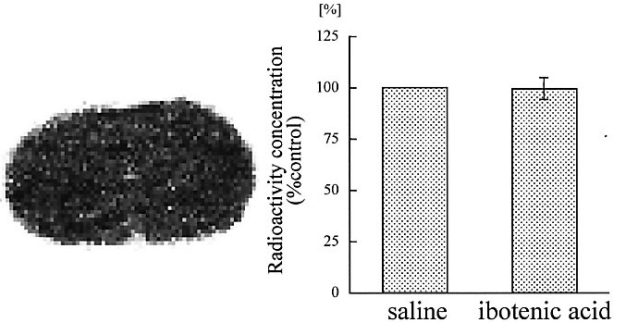


Fig. 4 Autoradiogram of cerebral blood flow measured with [^{14}C]iodoantipyrine. No significant alterations in blood flow were observed in the lesioned striatum. Values are expressed as relative radioactivity levels in the right striatum and left striatum (n = 3).

striatum (lesioned side) and left striatum (control side).

Statistical analysis

Statistical analysis was performed by Student's paired t-test.

RESULTS

The tissue distribution of [^{11}C]DMBA in mice is shown in Table 1. [^{11}C]DMBA was rapidly distributed into the brain, heart and lungs, and considerable long-term retention in the brain was observed. The level of unmetabolized [^{11}C]DMBA in the plasma was very low even at 1 min post injection of the tracer, and thereafter, it further declined along a single exponential curve. The radiochromatograms of the plasma samples are shown in Figure 2. More than 75% of the total radioactivity in plasma

collected 30 min post injection of the tracer was attributable to polar metabolites. Kinetic analysis using the [^{11}C]DMBA level in the plasma as the input function was performed, and the estimated distribution volumes in the brain, heart and lungs were 6.05, 3.95 and 7.35 respectively. The autoradiogram of [^{11}C]DMBA in rat brains lesioned revealed a significant reduction in the accumulation of [^{11}C]DMBA in the lesioned striatum (right side) (Fig. 3). On the other hand, regional cerebral blood flow in the right striatum (lesioned side) was not significantly altered as compared with that in the left striatum (control side) (Fig. 4).

DISCUSSION

[^{11}C]DMBA has a similar chemical structure to that of [^{11}C]methamphetamine. As previously reported, [^{11}C]MAMP was rapidly distributed into both mouse[8] and pig brain, and retained in tissue in contrast to β,β-difluoromethamphetamine. This suggested an important role of ionic interactions between basic amines and the brain tissue. [^{11}C]DMBA also rapidly entered into the mouse brain, and considerable long-term retention of its radioactivity in the brain was observed. These results suggest that ionic interactions might also be important for the uptake of this amine into the brain because of its high pKa (about 9.0). However, the elimination rate of [^{11}C]DMBA from the mouse brain was faster than that of [^{11}C]MAMP, which indicated that the affinity of [^{11}C]DMBA for this binding site is weaker than that of [^{11}C]MAMP. Metabolite analysis using HPLC revealed rapid metabolism of this amine in the peripheral tissues. As shown in Figure 2, the main labeled metabolite was polar, and therefore, the permeability of labeled metabolite through the blood brain barrier is likely to be very poor.

The most important finding in this study is that a significant reduction in the uptake of [^{11}C]DMBA in the right striatum lesioned with ibotenic acid was observed, as shown in Figure 3. However, the regional cerebral blood flow was not significantly altered in the striatum lesioned with ibotenic acid. The current observation on the cerebral blood flow in the lesioned striatum is consistent with previous reports, which indicated that there were almost no changes, or only a slight decrease of blood flow in the lesioned striatum of rat brain.[9] The brain uptake of [^{11}C]DMBA is considered to be affected mainly by two factors; cerebral blood flow and a binding component in the brain. The results show that the [^{11}C]DMBA binding component level in the brain is significantly decreased by lesioning with ibotenic acid. Preliminary experiment on the *in vitro* autoradiogram also lends support to our hypothesis that the levels of binding components for basic amines in the brain were decreased by lesioning with ibotenic acid (data not shown). The microinjection of ibotenic acid into the rat striatum has been reported to cause loss of neurons and induce glial reaction, but no damage to local microvessels,[10] and it has been used in an animal model for Huntington's disease. This supports our hypothesis that the binding components for basic amines in the brain exist in neural cells rather than glial cells. The results of several clinical studies on the brain uptake of [^{123}I]iodoamphetamine (IMP) in patients with glioma also support this hypothesis.[11] Nishizawa et al.[12] reported that increased IMP uptake was observed in tumors in the early images obtained within 20 min, which was followed by a rapid washout; this indicated increased blood flow to the tumor. Significant decrease of IMP uptake in primary brain tumors, regardless of increase or decrease in blood flow has also been reported.[5] These findings suggest that [^{11}C]DMBA has potential as a radiotracer for detection of neural cells in the brain.

Many clinical studies on brain perfusion in neurological diseases and other types of disease have been performed using IMP SPECT. Some reports have suggested the importance of the late images of IMP uptake. For example, increased accumulation of IMP in delayed images was observed in non-Hodgkin's lymphoma of the central nervous system.[13] An altered retention mechanism of IMP in a case of traumatic epilepsy has also been reported. Kawamura et al.[14] reported that delayed IMP SPECT images could be useful in patients with epileptic lesions that cannot be detected in the early images of IMP SPECT. Several reports have also indicated the importance of delayed IMP images in cerebrovascular disease,[15,16] although Steinling et al.[17] reported that delayed redistribution of IMP did not seem to be associated with neuronal metabolic activity. The brain uptake and kinetics of IMP seem to be affected by both cerebral blood flow and binding. It may be of some value to estimate the binding process of amines in the brain by PET.

In conclusion, [^{11}C]DMBA holds potential as a prototype tracer for estimation of the binding process of amines in the brain.

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