Annals of Nuclear Medicine Vol. 9, No. 3, 137–142, 1995

Brain uptake and metabolism of [1-^{11}C]octanoate in rats: Pharmacokinetic basis for its application as a radiopharmaceutical for studying brain fatty acid metabolism

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The uptake of octanoate in rat brain and its metabolism were investigated by means of intravenously injecting [1-^{11}C] or [1-^{14}C]octanoate as a tracer. The radioactivity in the cerebrum was increased by an injection of [1-^{11}C]octanoate, and reached its peak level (0.33% ID/g) in about 2 to 5 min, and then decreased slowly. The cerebrum-to-blood ratio of the radioactivity increased with time over a period of 30 min. At 30 sec, [1-^{11}C]octanoate that remained unchanged in the cerebrum accounted for only 8% of the total radioactivity, in spite of there being about 90% in the blood. By means of an injection of [1-^{14}C]octanoate, more than 70% of the total radioactivity in the cerebrum was found to be attributable to radiolabeled glutamate and glutamine at each time point measured between 30 sec and 30 min. The results show that [1-^{11}C]octanoate enters rat brain easily and is trapped in the cerebrum, probably in the form of glutamate and glutamine, and the usefulness of [1-^{11}C]octanoate as a radiopharmaceutical for studying brain fatty acid metabolism by positron emission tomography is therefore suggested.

Key words: [1-^{11}C]octanoate, radiopharmaceutical, brain, metabolism, rat

INTRODUCTION

IT IS WELL KNOWN that glucose is the predominant fuel for brain activity and that the quantities of other substrates, such as fatty acids and amino acids, oxidized by the brain are small relative to glucose.[1] On the other hand, it has been reported that the brain has enzymes for β-oxidation of fatty acids[2] and that ketone bodies formed from fatty acids are important substrates for energy metabolism during prolonged fasting.[3] Recently, Auestad N et al.[4] and Edmond J et al.[5,6] showed that the astrocytes play important roles in β-oxidation of fatty acids and ketogenesis in view of the results of studies with primary cultures of astrocytes. The relationship between brain functions and fatty acid metabolism is now one of the most interesting subjects in biology and medicine.

Studies with ^{14}C-labeled octanoic acid, an 8-carbon monocarboxylic saturated fatty acid, showed that this compound readily enters the brain[7,8] and acetyl-CoA from the first acetyl moiety split off by β-oxidation is rapidly metabolized to glutamine through the tricarboxylic acid (TCA) cycle and a small pool of glutamate.[9] The usefulness of [1-^{14}C]octanoate as a marker of cerebral blood flow and energy metabolism was also proposed as a result of autoradiographic studies.[10] These studies suggest that octanoate labeled with positron emitting nuclides is also applicable as a radiopharmaceutical for studying brain functions with positron emission tomography (PET).

In this regard, several studies on fatty acids labeled with positron emitting nuclides have been reported. [1-^{11}C]Octanoate was synthesized and its biodistribution was examined in animals.[11–13] Nagatsugi F et al.[14] synthesized 8-[^{18}F]fluorooctanoic acid and its related compounds and showed their biodistribution and brain uptake in rats. Recently, Ishiwata K et al.[15] evaluated the potential of [1-^{11}C]hexanoate (^{11}C-HA) as a radiopharmaceutical for assessing fatty acid metabolism in the myocardium and brain by means of biodistribution studies in mice. They confirmed that ^{11}C-HA is taken up into the brain rather more efficiently than [1-^{14}C]acetate or [1-^{14}C]palmitate. They also indicated that ^{11}C-HA is

Received March 29, 1995, revision accepted May 17, 1995.

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metabolized to ^{11}C-acetyl-CoA and then degraded further to $^{11}CO_2$ via the TCA cycle. But the brain uptake and metabolism of [1-^{11}C]octanoate remain to be elucidated.

The present study describes the brain uptake and metabolism of [1-^{11}C]octanoate in rats to provide a pharmacokinetic basis for its application as a PET tracer for studying brain fatty acid metabolism.

MATERIALS AND METHODS

[1-^{11}C]Octanoate and [1-^{14}C]octanoate

[1-^{11}C]Octanoic acid was synthesized by a Grignard reaction of $^{11}CO_2$ with heptyl magnesium bromide,[12,13] in an automated synthesis apparatus (Yajima K et al., J. Automatic Chem., in press). Briefly, $^{11}CO_2$ was produced in an ultracompact cyclotron (CYPRIS HM-18; Sumitomo Heavy Industries Co. Ltd., Tokyo, Japan) by the ^{14}N(p, α)^{11}C reaction in a N_2 gas target. The ^{11}C-labeled CO_2 was then introduced into a reactor, reacted with the Grignard reagent, and the product was hydrolyzed with hydrochloric acid. [1-^{11}C]Octanoic acid thus produced was purified by high performance liquid chromatography (HPLC) on a reverse-phase column (YMC PACK ODS-A, 250 mm × 20 mm i.d. or ODS-AQ, 250 mm × 10 mm i.d.; YMC Co. Ltd., Kyoto, Japan) with CH_3CN/0.012 N HCl (50 : 50, v/v). The radiochemical purity of the [1-^{11}C]octanoic acid obtained was found to be > 95% by means of HPLC on a reverse-phase column (YMC PACK ODS-AQ, 250 mm × 4.6 mm i.d. ; YMC Co. Ltd.) with CH_3CN/0.015 N HCl (60 : 40, v/v), and the specific activity was > 46 GBq/μmol at the end of formulation.

[1-^{14}C]Octanoate (radiochemical purity: > 99%, specific activity: 2.0 GBq/mmol) was obtained from NEN Research Products (Du Pont Co. Ltd., Boston, MA).

Animal Study

Male Sprague-Dawley rats (251–341 g; Charles River Japan Co. Ltd., Yokohama, Japan) were used. The rats were fed laboratory chow (CE-2; CLEA Japan, Tokyo, Japan) and had free access to water.

[1-^{11}C]Octanoate in saline-7% $NaHCO_3$ mixture or [1-^{14}C]octanoate in saline was injected intravenously into the tail vein of rats under light ether anesthesia. The doses of [1-^{11}C]octanoate and [1-^{14}C]octanoate injected were < 2 nmol/m*l* and ca. 2.5 μmol/m*l* per 1 kg body weight, respectively. At designated time intervals, the animals were sacrificed by decapitation under light anesthesia and their organs were removed.

Measurement of radioactivity

The ^{11}C-radioactivity in tissues and other samples was measured with a well-type scintillation counter (1480 WIZARD™3″; Wallac Co. Ltd., Turku, Finland). The ^{14}C-radioactivity in blood and tissues was determined with a liquid scintillation counter (LSC-3500; Aloka Co. Ltd., Tokyo, Japan) after combustion with a sample oxidizer (Model 307; Packard Instrument Co. Ltd., Meriden, CT). The ^{14}C-radioactivity in other samples was counted with the liquid scintillation counter.

Biodistribution in rats

The tissue and blood samples were weighed, and the radioactivity was determined. Results were expressed as % injected dose/g tissue weight (% ID/g).

Analysis of [1-^{11}C]octanoate in the cerebrum and blood

The head samples were frozen in liquid nitrogen and the blood samples were cooled in an ice bath immediately after decapitation. The frozen cerebrums were then removed and pulverized. The pulverized cerebrum (ca. 200 mg) and blood (0.5 m*l*) samples were homogenized with 80% MeOH (2 m*l*) and MeOH (2 m*l*), respectively, and then centrifuged (1800 × g, at 4°C) for 10 min. The ^{11}C-radioactivity in the supernatant was analyzed by HPLC on a reverse-phase column (YMC PACK ODS-AQ, 250 mm × 4.6 mm i.d.; YMC Co. Ltd.) with CH_3CN/0.01 N HCl (50 : 50, v/v) at a flow rate of 1.0 m*l*/min. The retention time of octanoic acid in this system was 12 min.

Analysis of [1-^{14}C]octanoate and its metabolites in the cerebrum and blood

The unchanged [1-^{14}C]octanoate in the cerebrum and blood were determined by HPLC with the same method as that used in the analysis of [1-^{11}C]octanoate with a slight modification. The radiolabeled metabolites, ^{14}C-labeled glutamate and glutamine, in the cerebrum were also determined by HPLC with the same method. In this case, however, the elution was done with CH_3CN/0.01 N HCl (1 : 99, v/v) containing 5 mM octanesulfonic acid (PIC B-8 reagent; Waters Co. Ltd., Milford, MA). The retention times of glutamine and glutamate in this system were 13 and 17 min, respectively.

RESULTS

Biodistribution of [1-^{11}C]octanoate in rats

The tissue distribution of [1-^{11}C]octanoate in normal rats is shown in Table 1. The radioactivity was cleared rapidly from the blood. The highest concentrations of radioactivity were observed in the heart and kidneys at the first sampling point of 30 sec, and then the concentrations decreased rapidly with time. The radioactivity in the liver reached a peak at 2 min after injection and thereafter maintained the highest concentration among the tissues examined. In the cerebrum, the radioactivity reached the maximum (0.33% ID/g) around 2 to 5 min after injection and then decreased slowly. The time-radioactivity curve in the cerebellum was similar to that in the cerebrum. The cerebrum-to-blood ratio of the radioactivity continued to increase with time, and exceeded 1 at 15 and 30 min after injection.

Table 1 Biodistribution of the radioactivity in rats after an intravenous injection of [1-^{11}C]octanoate

Tissue	Time (min)				
	0.5	2	5	15	30
Cerebrum	0.25 ± 0.06	0.33 ± 0.06	0.33 ± 0.03	0.26 ± 0.04	0.22 ± 0.02
Cerebellum	0.27 ± 0.06	0.32 ± 0.07	0.32 ± 0.03	0.27 ± 0.04	0.22 ± 0.02
Heart	2.49 ± 0.41	1.38 ± 0.36	0.41 ± 0.05	0.19 ± 0.04	0.17 ± 0.01
Lung	1.34 ± 0.15	0.84 ± 0.20	0.65 ± 0.15	0.45 ± 0.06	0.36 ± 0.01
Liver	1.73 ± 0.33	2.06 ± 0.41	1.55 ± 0.27	0.98 ± 0.19	0.58 ± 0.08
Kidney	2.05 ± 0.25	1.74 ± 0.07	0.97 ± 0.29	0.57 ± 0.09	0.41 ± 0.04
Blood	1.51 ± 0.97	0.58 ± 0.03	0.34 ± 0.02	0.23 ± 0.03	0.20 ± 0.02
Cerebrum/Blood*	0.26 ± 0.22	0.57 ± 0.10	0.97 ± 0.03	1.13 ± 0.08	1.19 ± 0.21

Data are the mean ± SD of 3 to 4 animals (% ID/g).
*Cerebrum to blood ratio.

Table 2 Compositions of the unchanged [1-^{11}C]octanoate and its radiolabeled metabolites in the cerebrum and blood of rats after an intravenous injection of [1-^{11}C]octanoate

Tissue	Time (min)	Total radioactivity	Octanoate	Metabolites
Cerebrum	0.5	0.39 ± 0.11 (100)	0.03 ± 0.01 (7.7)	0.36 ± 0.10 (92.3)
	2	0.38 ± 0.12 (100)	< 0.01 (—)	0.38 ± 0.12 (—)
Blood	0.5	1.58 ± 0.38 (100)	1.38 ± 0.41 (87.3)	0.21 ± 0.21 (13.3)
	2	0.44 ± 0.06 (100)	0.17 ± 0.04 (38.6)	0.27 ± 0.06 (61.4)

Data are the mean ± SD of 3 animals (% ID/g).
Values in parentheses denote the percentage of the total radioactivity.
—, not calculated.

Table 3 Time-courses of the radioactivity and compositions of the unchanged [1-^{14}C]octanoate and its radiolabeled metabolites in the cerebrum and blood of rats after an intravenous injection of [1-^{14}C]octanoate

Tissue	Time (min)	Total radioactivity	Octanoate	Metabolites
Cerebrum	0.5	0.29 ± 0.06 (100)	0.02 ± 0.00 (6.9)	0.28 ± 0.06 (96.6)
	2	0.37 ± 0.02 (100)	< 0.01 (—)	0.36 ± 0.01 (—)
	5	0.29 ± 0.02 (100)	N.D.	N.D.
	15	0.26 ± 0.05 (100)	N.D.	N.D.
	30	0.17 ± 0.03 (100)	N.D.	N.D.
Blood	0.5	1.13 ± 0.15 (100)	1.00 ± 0.19 (88.5)	0.13 ± 0.04 (11.5)
	2	0.38 ± 0.03 (100)	0.14 ± 0.01 (36.8)	0.24 ± 0.02 (63.2)
	5	0.19 ± 0.02 (100)	N.D.	N.D.
	15	0.13 ± 0.02 (100)	N.D.	N.D.
	30	0.15 ± 0.01 (100)	N.D.	N.D.

Data are the mean ± SD of 3 animals (% ID/g).
Values in parentheses denote the percentage of the total radioactivity.
—, not calculated. N.D., not determined.

Unchanged [1-^{11}C]octanoate in the cerebrum and blood

Table 2 shows the compositions of unchanged [1-^{11}C]octanoate and the labeled metabolites in the cerebrum and blood. The unchanged [1-^{11}C]octanoate accounted for only 8% of the total radioactivity in the cerebrum in contrast to about 90% in the blood at 30 sec, the first sampling point.

^{14}C-Radioactivity and unchanged [1-^{14}C]octanoate in the cerebrum and blood

Table 3 shows the time-courses of the radioactivity and the compositions of unchanged [1-^{14}C]octanoate and the labeled metabolites in the cerebrum and blood after an intravenous injection of [1-^{14}C]octanoate. The radioactivity was cleared rapidly from the blood. In the cerebrum, it

Table 4 Compositions of the unchanged [1-^{14}C]octanoate and its radiolabeled metabolites, ^{14}C-labeled glutamate and glutamine, in the cerebrum of rats after an intravenous injection of [1-^{14}C]octanoate

Time (min)	Total radioactivity	Octanoate	Glutamate	Glutamine	Others
0.5	0.29 ± 0.06	0.02 ± 0.00	0.06 ± 0.02	0.16 ± 0.02	0.06 ± 0.02
	(100)	(6.9)	(20.7)	(55.2)	(20.7)
2	0.37 ± 0.02	< 0.01	0.10 ± 0.01	0.21 ± 0.01	0.06 ± 0.02
	(100)	(—)	(27.0)	(56.8)	(16.2)
5	0.29 ± 0.02	< 0.01	0.09 ± 0.00	0.15 ± 0.02	0.05 ± 0.01
	(100)	(—)	(31.0)	(51.7)	(17.2)
15	0.26 ± 0.05	< 0.01	0.09 ± 0.02	0.12 ± 0.02	0.05 ± 0.01
	(100)	(—)	(34.6)	(46.2)	(19.2)
30	0.17 ± 0.03	< 0.01	0.06 ± 0.00	0.06 ± 0.02	0.05 ± 0.01
	(100)	(—)	(35.3)	(35.3)	(29.4)

Data are the mean ± SD of the 3 animals (% ID/g).
Values in parentheses denote the percentage of the total radioactivity.
—, not calculated.

reached a peak (0.36% ID/g) at 2 min after injection and then decreased slowly. The unchanged [1-^{14}C]octanoate in the cerebrum was also as low as 7% of the total radioactivity at 30 sec, whereas in the blood the unchanged [1-^{14}C]octanoate accounted for about 90% of the total radioactivity.

Metabolites of [1-^{14}C]octanoate in the cerebrum

Results of the analysis of the metabolites of [1-^{14}C]octanoate, ^{14}C-labeled glutamate and glutamine, in the cerebrum are shown in Table 4. The radiolabeled glutamate and glutamine accounted for more than 70% of the total radioactivity in the tissue at each time point of measurement (30 sec to 30 min).

DISCUSSION

In rats, after an intravenous injection of [1-^{11}C]octanoate, the peak concentration of the radioactivity in the cerebrum (0.33% ID/g, Table 1) was comparable to the estimated value (0.34% ID/g) based on the assumption that the injected radioactivity is distributed uniformly throughout the rat body. This result suggests that [1-^{11}C]octanoate readily enters the brain, and this is consistent with the high brain uptake index of [1-^{14}C]octanoate reported by Oldendorf WH et al.[7] In addition, the clearance of the radioactivity from the brain was slower than that from the blood (Table 1), indicating that some trapping may occur in the brain. This trapping of radioactivity in the brain seems to be mainly attributable to the metabolites of [1-^{11}C]octanoate, possibly as labeled glutamate and glutamine, because unchanged [1-^{11}C]octanoate was a minor component in the cerebrum (Table 2) and in view of the findings on [1-^{14}C]octanoate (Table 4).

It is difficult to examine the metabolic fate of a compound labeled with ^{11}C because of the short half-life of ^{11}C ($t_{1/2}$ = 20 min). Therefore, ^{14}C-labeled compounds are generally used as an alternative. However, a much higher dose is needed in experiments with ^{14}C-labeled compounds than with ^{11}C-labeled compounds (ca. 2.5 μmol/kg for ^{14}C-octanoate vs. less than 2 nmol/kg for ^{11}C-octanoate in the present study), because the specific activities of ^{14}C-labeled compounds are generally much lower than those of ^{11}C-labeled compounds. Accordingly, it is not always justifiable to directly compare the results obtained with ^{14}C-labeled compounds with those obtained with ^{11}C-labeled ones. The results in Tables 1 and 3 show, however, that the time-courses of radioactivity in the cerebrum and blood of [1-^{14}C]octanoate-injected rats were quite similar to those of [1-^{11}C]octanoate-injected rats, although the dose of [1-^{14}C]octanoate was more than 1000-fold as large as that of [1-^{11}C]octanoate. Moreover, there was also no significant difference in the composition of unchanged octanoate and metabolites in the cerebrum and blood between the rats given [1-^{11}C]octanoate and [1-^{14}C]octanoate (Tables 2 and 3). These results indicate that the dispositions of [1-^{11}C]octanoate and [1-^{14}C]octanoate in the rat cerebrum and blood are similar, regardless of the differences in specific activities and doses of the two tracers within the range used.

The brain uptake of radioactivity after an intravenous injection of [1-^{11}C] or [1-^{14}C]octanoate was a little less than that reported by Rowley H et al.[10] As the labeled compounds were administered under light anesthesia in the present experiment, the difference may be partly attributed to the decrease in cerebral blood flow and energy metabolism and the changes in glial metabolism caused by the anesthesia.

In the cerebrum, the radiolabeled glutamate and glutamine accounted for more than 70% of the total radioactivity at each time point of measurement (30 sec to 30 min) after an intravenous injection of [1-^{14}C]octanoate. This result agrees with that reported by Cremer JE et al.[9]

They indicated from the experiment with [1-^{14}C]octanoate that the rapid metabolism of octanoate into glutamate and glutamine rather than its utilization for lipogenesis is the major metabolic pathway in the cerebrum. The metabolism of octanoate into glutamate and glutamine may therefore be responsible for the retention of radioactivity in the cerebrum of rats after an intravenous injection of [1-^{14}C]octanoate, and the retention of ^{11}C-radioactivity in rat cerebrum after an intravenous injection of [1-^{11}C]octanoate may be explained by the same mechanism as that with [1-^{14}C]octanoate. It should be noted that CO_2 is produced during the metabolism of octanoate, but no significant amount of [1-^{14}C]octanoate-derived volatile ^{14}C-metabolite was detected in rat cerebrum during the time period of the experiment (data not shown), a relatively slow catabolism of octanoate towards CO_2 production in this tissue thus being suggested.

The present results show that [1-^{11}C]octanoate readily enters the brain and is trapped in this tissue, which indicates the usefulness of [1-^{11}C]octanoate as an imaging agent for studying brain activity *in vivo*. In this regard, the potential use of [1-^{11}C]octanoate as a marker for studying glutamate and/or glutamine in the cerebrum is expected from the metabolic characteristics of octanoate in this tissue. Moreover, [1-^{11}C]octanoate may be used to image astrocytes, because it is known that astrocytes, but neither oligodendrocytes nor neurons, are capable of metabolizing octanoate by β-oxidation[5,6] and that glutamine synthetase, an enzyme which catalyzes the conversion of glutamate to glutamine, is localized predominantly in astrocytes.[16] Thus, [1-^{11}C]octanoate may be used as a radiopharmaceutical for studying brain functions, such as functional disorder due to brain lesions. Regarding the usefulness of ^{11}C-fatty acids other than [1-^{11}C]octanoate, [1-^{11}C]acetate has been used as a tracer of the TCA cycle flux and thus overall oxidative metabolism in the myocardium.[17] In addition, Ishiwata K et al.[15] suggested the potential use of [1-^{11}C]hexanoate for imaging the oxidation process in the myocardium and brain. Referring to these ^{11}C-fatty acids, [1-^{11}C]octanoate also seems to be available for measuring the oxidative metabolism in the brain. However, this is rather unlikely because of the slow clearance of radioactivity from the brain owing to the presence of a metabolic route into glutamate and glutamine in this tissue.

The present study revealed high initial myocardial uptake of radioactivity (heart-to-blood ratio: 1.6 and 2.4 at 30 sec and 2 min, respectively) in rats after an intravenous injection of [1-^{11}C]octanoate. In this case also, the usefulness of this compound as a marker for cardiac functions is considered.

In this paper, we showed the usefulness of [1-^{11}C]octanoate for studying brain fatty acid metabolism, but it is necessary to know the scope and limitation of the application of this radiopharmaceutical for studies on cerebral and myocardial functions and diagnosis of the diseases. The establishment of such applications must await further investigations.

ACKNOWLEDGMENTS

This work was supported in part by a grant from the 'research and development programs for next-generation spinhead technologies' of the Japan Health Science Foundation. The authors are grateful to Dr. T. Omae, President of the National Cardiovascular Center, for supporting this work. We would like to thank Mr. M. Yamada and Mr. N. Ejima for technical assistance. We also thank Takeda Chemical Industries Co., Ltd. and T.N. Technos. Co., Ltd. for supporting this work.

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