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[^{14}C]Benzyl acetate is a potential radiotracer for the measurement of glial metabolism in the rat brain

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Abstract

In order to develop a suitable radiotracer for the measurement of glial metabolism, we synthesized four different types of ester derivatives of [^{14}C]acetate, namely, [^{14}C]phenyl acetate, [^{14}C]*para*-nitrophenyl acetate, [^{14}C]2,4-dinitrophenyl acetate and [^{14}C]benzyl acetate ([^{14}C]BA), and evaluated their potencies in rats. Among the derivatives, the highest brain uptake at 30 s postinjection was observed for [^{14}C]BA, which was more than 23 times higher than that of [^{14}C]acetate itself. A long-term retention of [^{14}C]BA radioactivity in the brain was observed, whereas rapid clearance of radioactivity was seen in the heart. [^{14}C]BA was rapidly hydrolyzed in the intact rat brain, and less than 5% of radiolabeled parent was observed 1 min after the injection. Radiochemical analysis using thin-layer chromatography revealed that [^{14}C]BA was rapidly converted to [^{14}C]glutamine and [^{14}C]glutamate in the cortex within 10 min after injection. Furthermore, the uptake of [^{14}C]BA was significantly decreased following microinjection of fluorocitrate, a selective glial toxin. These results strongly suggest that [^{14}C]BA may be a useful radiotracer for the measurement of glial metabolism in the intact rat brain.

Keywords: Phenyl acetate derivatives; Benzyl acetate; Glial metabolism; Brain; In vivo

1. Introduction

Exogenous acetate is taken up by glial cells through a monocarboxyl acid transporter-1 and converted to acetyl CoA, a substrate of the TCA cycle in glial cells. Previous reports have indicated that [^{3}H]acetate might be a potential marker of the glial metabolism [1] and that oxidative metabolism in the cerebral cortex could be investigated using [^{14}C]acetate [2]. Alteration in glial metabolism in Alzheimer's disease [3], depression [4] and animal model of schizophrenia [5], epilepsy [6] and manganism [7] has been suggested in recent studies. Important roles of glial metabolism in brain ischemia have also been reported [8–10]. Therefore, it seems to be very important to develop the new method for measurement of glial metabolism in the intact brain.

Recently, we found a marked reduction of [^{14}C]acetate uptake following microinjection of fluorocitrate, a selective glial toxin, into the striatum [11]. This result indicated that both transport and metabolic processes play important roles for [^{14}C]acetate uptake into glial cells. While radiolabeled acetate might be a useful tool for the measurement of glial metabolism in the intact brain, the brain uptake of [^{11}C]acetate is not sufficient for in vivo measurement with positron emission tomography (PET). Our animal experiment showed that the uptake of [^{14}C]acetate into the rat brain at 1 min postinjection was about one seventh of that of [^{18}F]fluoro-2-doxyglucose.

In order to improve the brain uptake, we designed and evaluated a new pro-radiotracer, [^{14}C]phenyl acetate ([^{14}C]PA) [12]. The uptake of [^{14}C]PA in the cerebral cortex at 1 min postinjection was about threefold higher than that of [^{14}C]acetate, indicating that [^{14}C]PA entered the brain by passive diffusion due to its high lipophilicity. [^{14}C]PA easily passed through the blood–brain barrier and then rapidly

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hydrolyzed to [^{14}C]acetate probably by carboxyl esterase in the rat brain. A significant decrease of [^{14}C]PA uptake was observed by inhibition of glial TCA cycle with fluorocitrate, in an analogous manner to [^{14}C]acetate. These results indicate that [^{14}C]PA might be useful as a prototype radiotracer for the measurement of glial metabolism in the intact brain.

The brain uptake and kinetics of the pro-radiotracer are influenced by several factors: lipophilicity of the tracer, cerebral blood flow and the hydrolysis rates both in peripheral tissue and the central nervous system. The hydrolysis rates of PA derivatives by carboxyl esterase can be changed by the modification of the phenyl ring. In this study, [^{14}C]PA derivatives, [^{14}C]*para*-nitrophenyl acetate ([^{14}C]pNPA), a typical carboxyl esterase substrate [13], [^{14}C]2,4-dinitrophenyl acetate ([^{14}C]dNPA) and [^{14}C]benzyl acetate ([^{14}C]BA) were synthesized and their potential as radiotracers for the measurement of glial metabolism was evaluated in rats (Fig. 1). The brain uptake, kinetics, distribution and hydrolysis of [^{14}C]pNPA, [^{14}C]dNPA and [^{14}C]BA in rat were measured and compared with [^{14}C]PA. [^{14}C]BA was found to be the most suitable radiotracer for glial metabolism mapping in the rat brain.

2. Materials and methods

2.1. Animals

Male Wistar rats (7–8 weeks old) were purchased from Japan SLC (Shizuoka, Japan). The animals were housed under a 12-h dark–light cycle and had continuous free access to food and water. All the animal experiments in this study were carried out with approval of the Institutional Animal Care and Use Committee, School of Allied Health Sciences, Osaka University.

2.2. Chemicals

[^{14}C]Acetate (specific radioactivity, 2.02 GBq/mmol) was purchased from Perkin Elmer Life Science (Boston, MA, USA). Phenol, acetic acid, benzylalcohol and *N*,*N*′-dicyclohexylcarbodiimide (DCC) were obtained from Nacalai Tesque, Inc. (Kyoto, Japan). *para*-Nitrophenol and 2,4-dinitrophenol were obtained from Wako Pure Chemicals (Osaka, Japan). Other chemicals were obtained from their respective manufacturers.

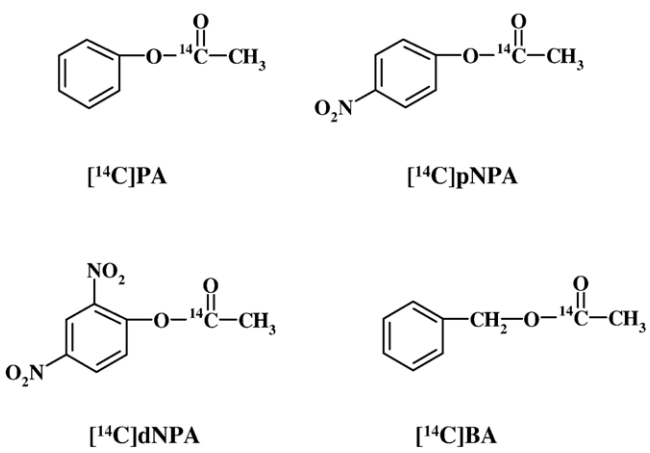

Fig. 1. Chemical structure of four ester derivatives of [^{14}C]acetate.

2.3. Preparation of [^{14}C]PA, [^{14}C]pNPA, [^{14}C]dNPA and [^{14}C]BA

[^{14}C]PA was synthesized by the same method as the previous report with minor modifications [12]. Briefly, [^{14}C]acetate (18.5 MBq, 3 mg of carrier acetate; 0.06 mmol) was allowed to react with phenol (0.11 mmol) and DCC (0.15 mmol) in 200 μl of dichloromethane at room temperature for 24 h. [^{14}C]PA was purified by thin-layer chromatography using dichloromethane as the solvent. *para*-Nitrophenol (0.07 mmol), 2,4-dinitrophenol (0.09 mmol) or benzylalcohol (1.1 mmol) was mixed in a glass tube with acetic acid (0.008–0.05 mmol), [^{14}C]acetate (3.7–18.5 MBq) and DCC (0.10–0.15 mmol) in dichloromethane. The mixture was stirred at room temperature for 18–24 h using the incubator (Iuchi Co. Ltd., Tokyo, Japan). After the reaction, [^{14}C]pNPA, [^{14}C]dNPA or [^{14}C]BA was purified by thin-layer chromatography using dichloromethane as the solvent. Ethyl acetate or ethanol was immediately added to the obtained sample, and the extract was passed through the glass filter. After the evaporation, 0.5–1.5 ml of ethanol was added and finally passed through Einmal-Filterhalter (0.2 μm; 7 bar max, pyrogen free).

Radiochemical purity of [^{14}C]BA was determined by thin-layer chromatography using dichloromethane as the solvent (R_f=0.8). The specific radioactivity of [^{14}C]BA was determined by calculating total radioactivity (18.5 MBq) and the amount of acetate (0.009 mmol of [^{14}C]acetate plus 0.05 mmol of carrier acetate). Radiochemical purities and the specific radioactivities of other labeled compounds were determined by the same method as that for [^{14}C]BA.

2.4. Brain uptake of [^{14}C]PA derivatives 30 s after the intravenous injection into rat

Rats (n=4) were intravenously injected with [^{14}C]pNPA, [^{14}C]dNPA or [^{14}C]BA and decapitated at 30 s postinjection. Cerebral cortex was quickly removed and weighed. Each sample was dissolved in 2 ml of tissue solubilizer (Soluen, Packard). After solubilization, the radioactivity of each sample was measured with a liquid scintillation counter (multipurpose scintillation counter: LS-6500, BECKMAN), and the radioactivity concentrations were expressed as a percentage of the total injected dose per gram of tissue.

2.5. In vivo hydrolysis of [^{14}C]PA, [^{14}C]pNPA, [^{14}C]dNPA and [^{14}C]BA in the brain or blood 1 min after the injection of each tracer into rats

Rats (n=3–4) were intravenously injected with [^{14}C]PA, [^{14}C]pNPA, [^{14}C]dNPA or [^{14}C]BA (185–370 kBq) and decapitated 1 min after the injection. The blood and brain