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^{11}C-Labeled Capsaicin and Its *in Vivo* Molecular Imaging in Rats by Positron Emission Tomography

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Abstract

Synthesis of ^{11}C-labeled capsaicin ([^{11}C]-2) was accomplished by *O*-[^{11}C]methylation with [^{11}C]-methyl iodide to a corresponding catechol precursor (1). *In vivo* whole-body distribution of [^{11}C]-2 in rats was investigated by positron emission tomography after intravenous injection. The results showed that [^{11}C]-2 accumulated in the brain and body surface, which was closely associated with the expression of transient receptor potential vanilloid 1.

Keywords

PET, Capsaicin, ^{11}C-Labeling, TRPV1, Blood-Brain Barrier

1. Introduction

Capsaicin, as an active compound found in red pepper, is pungent and produces an irritating sensation upon oral ingestion. Capsaicin belongs to the vanilloid family and is involved in various important biological activities related to human health, including thermal sensing, perspiration, and pain relief. Capsaicin acts via transient receptor potential vanilloid 1 (TRPV1) [1]-[3], which is a target of medicinal chemistry for drug development. However, the pathophysiological function of TRPV1 in disease is still unclear. Therefore, we synthesized ^{11}C-labeled capsaicin ([^{11}C]-**2**) for *in vivo* molecular imaging of capsaicin by positron emission tomography (PET) [4].

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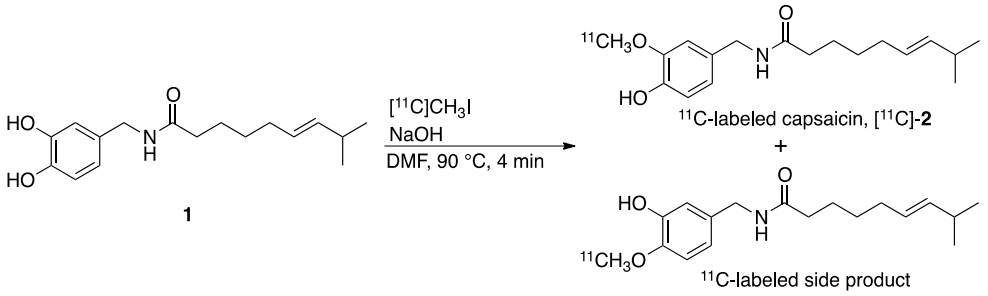
Specific radiochemical aspects of $^{11}C^{\#}$, such as a short radioactive half-life of 20.4 min, very small amount of available radionuclide (^{11}C-labeling precursor: normal level, 10 - 500 nmol), and high-energy radiation (γ-rays of 511 keV), impose severe restrictions on the synthesis of PET probes. In general, synthesis of a ^{11}C-labeled probe should be accomplished as much as possible within 2 half lives (*i.e.*, 40 min) because of the rapid radioactive decay of ^{11}C. This process includes the following steps: derivatization of a ^{11}C isotope produced by a cyclotron to an appropriate labeling precursor such as [^{11}C]methyl iodide ([^{11}C]CH_3I), ^{11}C-labeling of the target probe, chromatographic purification of the desired ^{11}C-labeled probe, and preparation of an injectable solution for an animal/human PET study. A remote-controlled synthesizer is also needed for the synthesis of the radiotracer to protect personnel from harmful radiation. In terms of an animal/human PET study, there is an absolute requirement for chemical synthesis of a ^{11}C-labeled PET probe with a radioactivity of at least several hundred MBq.

2. Results and Discussion

Considering such PET radiolabeling conditions and characteristics, we synthesized ^{11}C-labeled capsaicin ([^{11}C]-**2**) as a PET probe by *O*-[^{11}C]methylation using [^{11}C]CH_3I and a corresponding catechol precursor **(1)** at 90˚C for 4 min in the presence of NaOH (**Scheme 1**). High-performance liquid chromatography (HPLC) analysis of the reaction mixture showed two major ^{11}C-labeled products, the desired [^{11}C]-**2** and a side product [^{11}C]methylated at the para position (**Figure 1**).

[^{11}C]-**2** was isolated by semi-preparative HPLC. Then, an injectable saline solution containing [^{11}C]-**2** was prepared after filter sterilization. The total synthesis time was 36 - 37 min and the radioactivity of the injectable solution of [^{11}C]-**2** was up to 1.2 GBq. The specific radioactivity of [^{11}C]-**2** at the end of synthesis was 67 - 102 GBq/μmol. The chemical and radiochemical purities of the injectable solution were both >99%.

A [^{11}C]-**2** solution with a radioactivity of 65 MBq was intravenously injected via the tail vein of the rats. The injected mass corresponded to approximately 0.2 - 0.3 μg (0.65 - 1.0 nmol) of capsaicin. A dynamic PET scan of [^{11}C]-**2** in the brain and whole body of the rat was conducted for 90 min after injection of [^{11}C]-**2**. As shown in **Figure 2(a)** and **Figure 2(b)**, most of the [^{11}C]-**2** accumulated in the liver, intestinal tract, and urinary bladder. In addition, accumulation of [^{11}C]-**2** was observed on the body surface, which could indicate binding of [^{11}C]-**2**



Scheme 1. Synthesis of ^{11}C-labeled capsaicin by *O*-[^{11}C]methylation using [^{11}C]CH_3I.

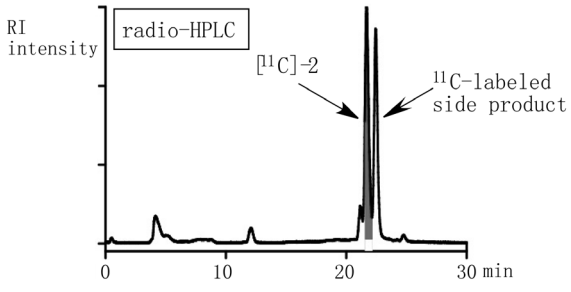
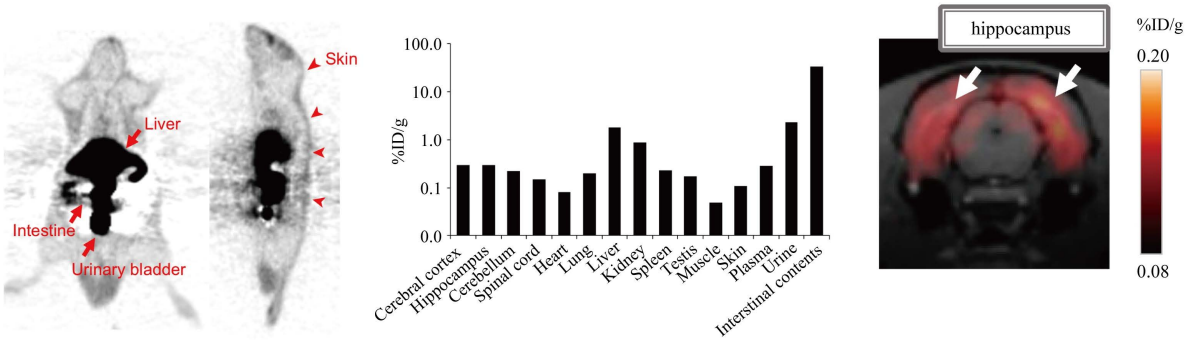


Figure 1. Preparative HPLC profile of the reaction mixture.

[#]In a typical experiment, the amount of [^{11}C]CH_3I as a ^{11}C-labeling precursor with radioactivity of 30 GBq and specific radioactivity of 500 GBq/μmol at the start of the probe synthesis is equivalent to 60 nmol.



(a) (b) (c)

Figure 2. PET imaging and tissue distribution analysis of rats injected with [^{11}C]-**2**. (a) Whole-body PET images were acquired from 5 to 90 min after injection of [^{11}C]-**2** (coronal plane in left side; sagittal plane in right side). High [^{11}C]-**2** accumulation was observed in the liver, intestine, urinary bladder, and body surface (arrows); (b) Tissue distribution of ^{11}C radioactivity in the rat. [^{11}C]-**2** uptake is shown as the percentage injected dose per gram of tissue (%ID/g), which represents the tissue radioactivity concentration (Bq/g) per injected radioactivity; (c) PET image overlaid on magnetic resonance image of the rat brain. The image shows relatively high uptake of [^{11}C]-**2** in the hippocampus.

to the TRPV1 receptor in the skin [1]-[3]. Interestingly, [^{11}C]-**2** was taken up into the brain through the blood-brain barrier (BBB), particularly in the hippocampus and cerebral cortex at 10 min post-injection (**Figure 2(b)** and **Figure 2(c)**). Such high-uptake regions in the brain can be considered as high TRPV1-expressing regions [5].

Thus, the PET images obtained were found to be in agreement with previous reports on the *in vitro* and *in vivo* biological activities of capsaicin [1]-[3]. Detailed analysis of the PET images is currently in progress and will be reported in due course.

3. Conclusion

Synthesis of ^{11}C-labeled capsaicin ([^{11}C]-**2**) was accomplished by *O*-[^{11}C]methylation using [^{11}C]CH_3I and the corresponding catechol precursor **(1)**. PET images of rats obtained using [^{11}C]-**2** showed not only possible evidence of accumulation in TRPV1-expressing regions but also BBB permeability of capsaicin. These encouraging results would promote further biological and medical studies on capsaicin. Based on [^{11}C]-**2** synthesis, we intend to synthesize various ^{11}C-labeled capsaicin analogs as PET probes to enable PET molecular imaging for both biologically important dietary constituents and drug development focusing on TRPV1.

4. Experimental

4.1. Animal Experiments

The animal experiments were approved by and performed in accordance with the guidelines of the Animal Care and Use Committee of RIKEN Kobe Institute (MAH21-13).

4.2. PET Imaging

A small animal PET scanner (microPET Focus220, Siemens Medical Systems) was used for the imaging experiments. Rats were anesthetized with 1.5% isoflurane prior to insertion of an indwelling catheter in the tail vein for tracer injection. During the scan, their body temperature was maintained at 37˚C by using a heating pad.

4.3. Chemical Synthesis

(*E*)-*N*-(3,4-dihydroxybenzyl)-8-methyl-6-nonenamide, catechol precursor (1):

According to a published procedure [6], **1** was prepared by condensation of 3,4-dihydroxybenzylamine and (*E*)-8-methyl-6-nonenoyl chloride in the presence of $NaHCO_3$ in a biphasic system of H_2O and $CHCl_3$.

Synthesis of (*E*)-*N*-(3-[^{11}C]methoxy-4-hydroxybenzyl)-8-methyl-6-nonenamide, ^{11}C-labeled capsaicin ([^{11}C]-2):

The [^{11}C]methylation in **Scheme 1** was conducted in a lead-shielded hot-cell operated by remote control. [^{11}C] carbon dioxide was produced by a ^{14}N(p,α)^{11}C reaction using a Sumitomo CYPRIS HM-12S cyclotron (Sumitomo Heavy Industries) and then converted to [^{11}C]CH_3I by treatment with lithium aluminum hydride followed by hydriodic acid using an original radiolabeling system for ^{11}C-labeling at RIKEN. The [^{11}C]CH_3I obtained was used for *O*-[^{11}C]methylation to the catechol precursor **(1)** as shown in **Scheme 1**. The [^{11}C]methylated products were assessed by an analytical HPLC system consisting of a Shimadzu HPLC system with a system controller (CBM-20A), online degasser (DGU-20A3), solvent delivery unit (LC-20AD), column oven (CTO-20AC), photodiode array detector (SPD-M20A), radioanalyzer (Aloka RLC-700), and analysis software (LC-Solution). The radioactivity was quantified with a CRC-15R (Capintec).

[^{11}C]CH_3I (approximately 30 GBq) was trapped in a solution of catechol precursor **(1)** (1 mg, 3.4 μmol) and NaOH (5 M aqueous solution, 6 μL, 30 μmol) in dimethylformamide (0.4 mL) at room temperature. The resulting mixture was heated at 90˚C for 4 min by hot air, and then neutralized with a mixed solution of aqueous HCl (6 M, 5 μL, 30 μmol) and aqueous CH_3CN (50%, 0.6 mL). The mixture was then injected into a preparative HPLC column (COSMOSIL 5C18 AR-II, 20 × 250 mm [Nacalai Tesque]). The mobile phase was first CH_3CN:CH_3OH:CH_3COONH_4 (30 mM aqueous solution) (5:30:65) from 0 to 1.5 min, and then CH_3CN:CH_3OH:CH_3COONH_4 (30 mM aqueous solution) (5 - 0:45 - 70:50 - 30) from 1.5 to 19 min, followed by CH_3OH:CH_3COONH_4 (30 mM aqueous solution) (70:30) from 19 to 30 min. The flow rate was 5.0 mL/min. UV detection was performed at 215 nm. The retention time of ^{11}C-labeled capsaicin, [^{11}C]-**2**, was 18.5 min. The yield of [^{11}C]-**2** determined by analytical radio-HPLC was 26% - 34% (n = 5), which was calculated by the peak area ratio of ^{11}C-containing product distributions. The desired fraction was collected in a flask and concentrated by removing the organic solvent under reduced pressure. The desired [^{11}C]-**2** was then diluted in a mixture of saline (2.5 mL), propylene glycol (0.11 mL), and Tween 80 (2 μL). The total synthesis time, including HPLC purification and radiopharmaceutical formulation, was 36 - 37 min. The radioactivity of the formulated injection solution was up to 1.2 GBq. The specific radioactivity was 67 - 102 GBq/μmol. The decay-corrected radiochemical yield of [^{11}C]-**2** was approximately 14% (n = 3), which was calculated on the basis of the radioactivity of [^{11}C]CH_3I trapped in the solution. [^{11}C]-**2** was identified by co-injection of commercially available non-radiolabeled capsaicin using an analytical HPLC column (COSMOSIL 5C18 AR-II, 4.6 × 150 mm [Nacalai Tesque]). The mobile phase was CH_3CN:0.1% aqueous H_3PO_4 (40:60). The flow rate was 1.0 mL/min. UV detection was performed at 254 nm. The retention time was 12.3 min. The chemical and radiochemical purities of the isolated [^{11}C]-**2** were both >99%.

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