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Preparation and biodistribution in mice of [^{11}C]carfentanil: a radiopharmaceutical for studying brain μ-opioid receptors by positron emission tomography

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A potent μ-opioid agonist, [^{11}C]carfentanil, was prepared by the methylation of carfentanil carboxylic acid with [^{11}C]methyl iodide in order to study brain μ-opioid receptors by positron emission tomography. Synthesis (including purification) was completed within 25 min and the radiochemical yield was approximately 40%. The radiochemical purity of the product was more than 99% and its specific activity was 3.7–7.4 GBq/μmol. Biodistribution studies performed in mice after intravenous injection showed a high brain uptake and rapid blood clearance, so a high brain/blood ratio of 1.5–1.8 was found from 5 to 30 min. Regional cerebral distribution studies in the mouse showed a significantly higher uptake of [^{11}C]carfentanil by the thalamus and striatum than by the cerebellum, with the radioactivity in the striatum disappearing more rapidly than that in the thalamus. Treatment with naloxone significantly reduced the uptake of [^{11}C]carfentanil by the thalamus and striatum. These results indicate that [^{11}C]carfentanil binds specifically to brain μ-opioid receptors.

Key words: [^{11}C]carfentanil, synthesis, biodistribution, opioid receptor, positron emission tomography

INTRODUCTION

WITH THE DISCOVERY of specific opioid receptors and subsequently of endogenous opioid peptides, considerable interest has been developed in the relationship of opioid receptor systems to pain control, psychiatric disorders, and neurodegenerative diseases.[1,2] Recently, positron emission tomography (PET) has been applied to the localization and quantification of opioid receptors in the central nervous system.[3–5]

PET studies of opioid receptors require the use of a radioligand labeled with a suitable positron-emitting radionuclide that possesses a very high affinity for the target receptors.[5–7] Cafentanil is a μ-subtype-selective opioid agonist which is over 7,000 times more potent than morphine[3,8–10] Accordingly, [^{11}C]carfentanil (Fig. 1) has been synthesized and used to study μ-opioid receptors in the human brain by PET.[3–5,11,12] However, in order to fully assess the usefulness of this radioligand for PET studies, more detailed information on its biodistribution is required, including the regional cerebral distribution.

In the present study, [^{11}C]carfentanil was prepared and its biodistribution, including the regional cerebral distribution as well as the effect of naloxone on the distribution pattern, was studied in mice because the regional cerebral localization of μ-opioid receptors is similar in mouse and human brains.[13,14]

MATERIALS AND METHODS

The sodium salt of carfentanil carboxylic acid was kindly provided by Dr. R. Dannals (The Johns Hopkins Medical Institutions, U.S.A.). Naloxone hydrochloride was purchased from Sigma. The other

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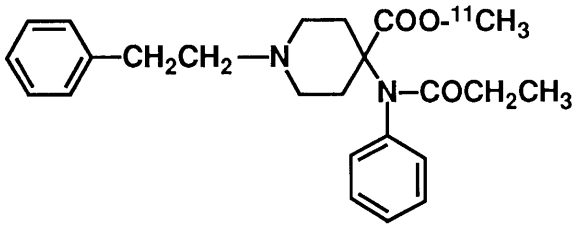

Fig. 1 Chemical structure of [^{11}C]carfentanil.

chemicals used were all of reagent grade. Male ddY mice were supplied by Japan SLC Co. Ltd.

Synthesis of [^{11}C]carfentanil

We synthesized [^{11}C]carfentanil according to the method of Dannals et al.[15]

[^{11}C]Carbon dioxide was produced via proton bombardment of nitrogen gas and the ^{14}N (p, α) ^{11}C reaction using an ultra-compact cyclotron (Sumitomo, Model 325), and was trapped in a solution of lithium aluminum hydride in tetrahydrofuran (THF). After evaporation of the THF, 54% hydroiodic acid was added, and the [^{11}C]methyl iodide produced was trapped in dimethylformamide (DMF) under a stream of nitrogen gas. 1 mg of cafentanil carboxylic acid sodium salt was then dissolved in 100 μl of DMF in a reaction vial, and 200 μl of DMF containing [^{11}C]methyl iodide was added to the vial. The vial was sealed and then the mixture was stirred at 35°C. After 5 min, the reacted mixture was purified with preparative HPLC on a 7.6×300 mm Lichrosorb RP-18 column eluted at 2.5 m*l*/min with methanol/0.1 M ammonium formate (7/3) (Rt=8.1 min for methyl iodide, Rt=15 min for carfentanil, and Rt=6.5 min for carfentanil carboxylic acid). The fraction corresponding to carfentanil was collected and evaporated to dryness. The residue was then dissolved in 3 m*l* of saline and 3.6 m*l* of 7% sodium bicarbonate solution, and filtered through a 0.22-μm filter. Radiochemical purity was determined by analytical HPLC on a 3.9×250 mm Lichrosorb RP-18 column eluted at 2 m*l*/min with methanol/0.1 M ammonium formate (7/3) (Rt=11 min for carfentamil). The specific activity was estimated from the U.V. absorbance at 254 nm.

Biodistribution in mice

A dose of 1.11 MBq (5 μg/kg) of [^{11}C]carfentanil was injected intravenously into male ddY mice weighing about 30 g. At various times after the injection, the mice were killed by decapitation, their organs were excised, and blood samples were collected by cardiac puncture. All samples were weighed and the radioactivity was counted in a well-type NaI scintillation counter. Results are presented as the % dose/g organ weight.

Regional cerebral distribution in the mouse

Male mice weighing an average of 30 g were injected intravenously with 0.1 m*l* of a solution of [^{11}C]-carfentanil (1.11 MBq, 5 μg/kg). At various times afterwards, the mice were killed by decapitation, and the various brain regions were dissected out on an ice-cold plate according to the method of Glowinski and Iversen.[16] The wet tissue samples were then weighed, and the radioactivity was determined in a well-type NaI scintillation counter. Results were calculated in terms of the % dose/g tissue weight.

Effect of naloxone on regional cerebral distribution in the mouse

Naloxone (1 mg/kg) was injected simultaneously with the radioligand into mice weighing an average of 30 g. The animals were killed 5 and 30 min after administration and the uptake of the radioligand by various brain regions was determined as described above.

RESULTS AND DISCUSSION

Preparation of [^{11}C]carfentanil

We synthesized [^{11}C]carfentanil by the methylation of carfentanil carboxylic acid with [^{11}C]methyl iodide. The labeling and purification procedures were completed within 25 min after the finish of [^{11}C]methyl iodide trapping and the radiochemical yield was approximately 38% based on the initial radioactivity of [^{11}C]methyl iodide. Radiochemical purity was comfirmed to be more than 98% by HPLC (Fig. 2). The specific activity of the product was 3.7–7.4 GBq/μmol at the end of the synthesis.

When *in vivo* PET studies of neuroreceptors are performed, high specific activity of the radioligand used is important both to achieve a specific distribution (low specific activity results in excessive nonspecific binding) and to avoid receptor occupancy levels high enough to cause pharmacological effects.[6,17,18] The specific activity obtained in this study was somewhat lower than the theoretical value (3.4×10^5 GBq/μmol), and this may have been due to contamination with carrier carbon-12 from the carbon dioxide in the target gas and methanol formed from the THF solution containing lithium aluminum hydride, as has been reported by some other groups.[13,18–20] However, it has also been reported that there exists a lower limit at which it is difficult to overcome the effect of the carrier containing the final ^{11}C-labeled product synthesized via [^{11}C]methyl iodide and that this limit is about 0.2 μmol.[19] Since the total amount of carrier contained by [^{11}C]carfentanil produced in this study was about 0.25 μmol, a shorter synthesis time and a higher initial ^{11}C radioactivity may be necessary to achieve a similar

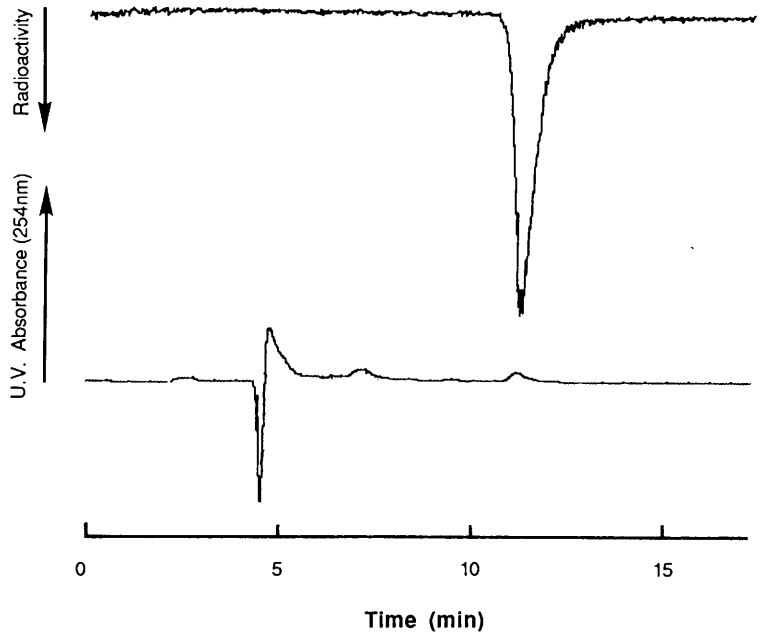

Fig. 2 HPLC elution profile of [^{11}C]carfentanil. HPLC conditions: column; Lichrosorb RP-18 (3.9×250 mm); mobile phase; methanol/0.1 M ammonium formate (7/3); flow rate; 2 ml/min.

Table 1 Biodistribution of [^{11}C]carfentanil in mice (% dose/g organ)

Organ	Time (min)			
	5	15	30	60
Blood	2.09 (0.17)	1.79 (0.39)	1.47 (0.27)	1.18 (0.14)
Liver	8.53 (0.47)	9.89 (1.15)	9.89 (3.48)	12.46 (1.39)
Kidney	14.66 (2.82)	9.03 (2.83)	6.97 (1.22)	5.88 (0.71)
Lung	6.43 (1.86)	3.86 (0.89)	2.86 (0.74)	1.90 (0.51)
Heart	3.91 (0.58)	2.35 (0.31)	1.83 (0.17)	1.22 (0.25)
Brain	3.73 (0.34)	2.85 (0.94)	2.13 (0.55)	1.28 (0.25)
Br/Bl*	1.79 (0.14)	1.54 (0.42)	1.46 (0.27)	1.09 (0.17)

Values are the mean (S.D.) for 4 animals.
* Brain/blood ratio of % dose/g organ.

specific activity to that reported in clinical studies (42 GBq/μmol).[3,15]

In vivo *studies*

Table 1 shows the distribution of [^{11}C]carfentanil in various organs of the ddY mouse. Radioactivity was cleared rapidly from the blood after injection. The brain showed a relatively high uptake of 3.7% dose/g at the initial sampling time (5 min), after which it declined steadily. The brain/blood ratio was high, ranging from 1.5–1.8 throughout the period from 5 to 30 min. High initial uptake was also observed in the lungs, kidneys, and liver, with the radioactivity in the former two organs clearing rapidly and that in the latter increasing further with time.

The distribution of [^{11}C]carfentanil radioactivity in various brain regions is shown in Table 2. Radioactivity was high in the thalamus and striatum, intermediate in the cortex, and low in the cerebellum. Accordingly, the ratios of radioactivity in the thalamus, striatum, and cortex to that in the cerebellum were 2.2–3.2, 1.9–2.3, and 1.4–1.7, respectively, at 5 to 30 min after injection. This marked regional variation in the distribution of [^{11}C]carfentanil agreed well with the results obtained by *in vitro* mapping of μ-opioid receptors.[13,14] Futhermore, treatment with naloxone, a μ-opioid antagonist,[20] significantly reduced the uptake of [^{11}C]carfentanil in the thalamus and striatum (73–74% inhibition for the thalamus and 62–82% for the striatum), so that the radioactivity in these two regions became almost the same as that in the cerebellum (Fig. 3).

Table 2 Regional cerbral distribution of [^{11}C]carfentanil in mice (% dose/g tissue)

Region	Time (min)			
	5	15	30	60
Striatum	4.53 (1.59)	2.01 (0.74)	1.28 (0.65)	0.50 (0.26)
Thalamus	3.98 (1.15)	2.56 (0.48)	1.81 (0.49)	0.98 (0.42)
Cortex	2.66 (0.98)	1.56 (0.34)	0.92 (0.13)	0.59 (0.12)
Cerebellum	2.04 (0.77)	1.08 (0.20)	0.55 (0.08)	0.55 (0.02)

Values are the mean (S.D.) for 4 animals.

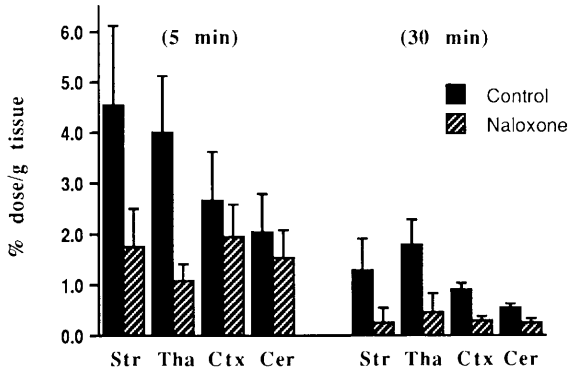

Fig. 3 Effect of naloxone on the regional distribution of [^{11}C]carfentanil in the mouse brain. Values are the mean (S.D.) for 4 animals. Str: striatum, Tha: thalamus, Ctx: cortex, Cer: cerebellum.

The time course of changes in radioactivity was different in the thalamus from that in the striatum, with the disappearance of striatal radioactivity being about twice as fast as that in the thalamus ($T_{1/2}$= 11.7 min for the striatum and 23.8 min for the thalamus) (Table 2). Since two subtypes of μ-receptors (μ_1 and μ_2) have recently been proposed to exist,[1,2,14,22] the differences in the distribution pattern of [^{11}C]-carfentanil by the thalamus and striatum might be related to the differential regional localization of μ-receptor subtypes.

In conclusion, the results of these *in vivo* regional cerebral distribution and competition studies indicated that [^{11}C]carfentanil binds specifically to brain μ-opioid receptors, offering a good basis for the application of this agent to clinical studies of such receptors.

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