Preparation of [11C]Caffeines from [11C]Methyl Iodide

III. 2. Preparation of [^{11}C]Caffeines from [^{11}C]Methyl Iodide

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Caffeine is one of the strongest and most popular stimulants such as ethanol and nicotine among naturally occurring compounds. It is well-known that caffeine has many pharmacological effects including inhibition of phospodiesterase[1] and binding adenosine receptor ligands[2] although in vivo behavior of caffeine has not been fully studied yet.

Our systematic study on the biological behavior of caffeine required a radioactive caffeine preferably with a high specific activity. Commercially available caffeines labeled with ^{3}H or ^{14}C, however, have rather low specific activities. As a result we have investigated the preparation of ^{11}C-labeled caffeines. They have already been synthesized by the N-[^{11}C]methylation of desmethyl derivatives of caffeine using [^{11}C]methyl iodide[3-5]. In our study we have labeled caffeine with ^{11}C at three different methyl groups starting from theobromine, paraxanthine and theophylline using high specific activity [^{11}C]methyl iodide for comparison of their *in vivo* behavior and metabolism. Our goal is the PET study with these positron emitting caffeines.

Materials and Methods

The radiosynthesis was carried out by the on-line [^{11}C]methylation described in our previous report[6]. The packing material in a reaction column was prepared as follows; each substrate (theobromine, paraxanthine and theophylline) was dissolved in an appropriate solvent, coated on an inert support (Flusin T or glass beads) by carefully removing the solvent with a rotary evaporator, and then mixed with Porapak Q (Waters) as thc adsorber for [^{11}C]methyl iodide.

[^{11}C]Methyl iodide was prepared from [^{11}C]carbon dioxide using the fully automated synthesis system (NKK Co., Japan). It was then trapped by the reaction column, cooled at -42°C with a dry ice-acetonitrile bath, of the automated on-line system. A 0.2 ml portion of dimethyl formamide (DMF) containing 25 mM NaOH was introduced into the column while cooled and the column was then heated at 80°C for 5 min. The reaction mixture was immediately injected onto a μBondapak C_{18} semi-preparative HPLC column (Waters, RCM 25×10), simply by switching the 6-way valve connected with the reaction column instead of a sample loop. The column was eluted with H_2O/methanol/acetic acid (70/29/1) at a flow rate

of 9.7 ml/min. The HPLC eluent was monitored with a u.v. detector (wavelength; 254 nm) and a small radiation sensor. Figure 1 shows a typical HPLC separation for the [7-*methyl*-^{11}C]caffeine preparation. The three starting substrates eluted between 6 and 8 min after the injection and the [^{11}C]caffeines at 9-10 min. In order to determine radiochemical yields, all the radioactivity, eluting from the HPLC column with the above solvent and also 40 ml of ethanol for washing the column after the product had been completely recovered, was collected in vials and each radioactivity was measured. Radiochemical purities and specific activities of [^{11}C]caffeines were determined by quantitative HPLC analysis using a NOVA-Pak C_{18} column (RCM 8×10, Waters) and a solvent system of H_2O/methanol/acetic acid (74/25/1) at a flow rate of 3.0 ml/min.

Results and discussion

Three ^{11}C-labeled caffeines, [1-*methyl*-^{11}C]caffeine, [3-*methyl*-^{11}C]caffeine and [7-*methyl*-^{11}C]caffeine, were successfully synthesized from theobromine, paraxanthine and theophylline, respectively, according to the reaction scheme shown in Fig. 2, and to our knowledge [3-*methyl*-^{11}C]caffeine was prepared for the first time. Table 1 lists their radiochemical yields and specific activities. The radiochemical yields of [3-*methyl*-^{11}C]caffeine and [7-*methyl*-^{11}C]caffeine were slightly improved by the present on-line [^{11}C]methylation method as expected, whereas the radiochemical yield of [1-*methyl*-^{11}C]caffeine was still low, probabl due to poor solubility of theobromine (approx. lg dissolves in a 2 liter portion of water). Therefore it is reasonable to expect that the optimization of reaction solvent and base concentration will improve this low radiochemical yield although the yield obtained by the present condition is practically high enough for subsequent use in biological or PET studies. In addition, both the radiochemical purity and specific activity werc also sufficiently high for our purpose.

References

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Table. 1. Synthetic results of [^{11}C] caffeines

	Radiochemical yield (%)	Specific activity (Ci/μmol : EOB)
[1-*methyl*-^{11}C]caffeine	27	3.1
[3-*methyl*-^{11}C]caffeine	64	3.9
[7-*methyl*-^{11}C]caffeine	68	6.7

Reaction conditions

Substrate amount: 50 mg, Solvent: 25 mM NaOH/DMF, Time 5 min, Temperature: 80 °C.

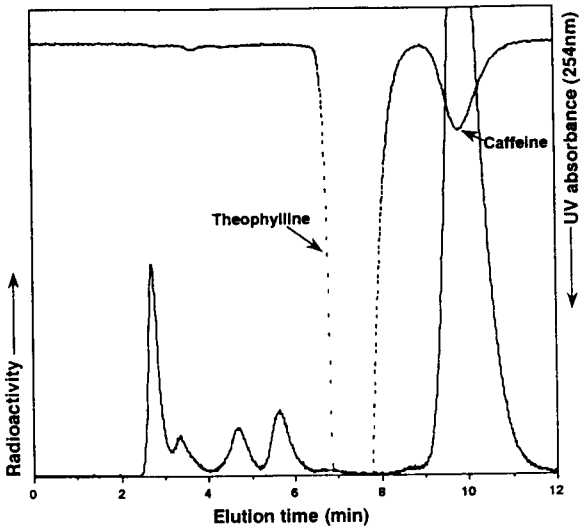

Fig. 1. A typical elution profile of semi-preparative HPLC for the [7-*methyl* -^{11}C] caffeine preparation

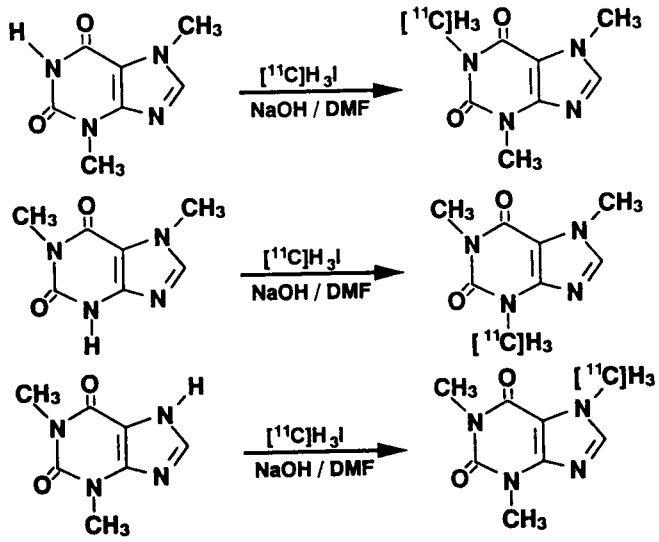

Fig. 2. Synthesist of three ^{11}C-labeled caffeines from [^{11}C] methyl iodide