Carbon-11-Forskolin: A Ligand for Visualization of the Adenylate Cyclase-Related Second Messenger System

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Signals of certain neurotransmitters and hormones are mediated by second messenger systems. One well-characterized second messenger system is the adenylate cyclase (AC)-related pathway, which catalyzes the receptor-mediated generation of cyclic adenosine monophosphate (cAMP), resulting in the activation of specific phosphorylating enzymes (1). Forskolin, a diterpene isolated from Coleus forskolii, specifically binds to the alpha subunit of stimulatory guanosine nucleotide binding protein (Gsα) and AC, which it then activates (2). Previously, we synthesized [11C]forskolin from 7-deacetylforskolin and [11C]acetic acid using dicyclohexycarbodiimide (DCC) (3, 4). In this study, [11C]forskolin, [11C]1-acetyl-7-deacetylforskolin, [11C]1,9-dideoxyforskolin and [11C]1-deoxyforskolin were synthesized by acetylation of the respective deacetyl-precursors using [11C]acetylchloride and dimethylaminopyridine. The 1- and 9-OH groups on the forskolin structure are critical for their ability to activate AC (5, 6). Therefore, we considered [11C]1-acetyl-7-deacetylforskolin, [11C]1,9-dideoxyforskolin and [11C]1-deoxyforskolin to be nonspecific forskolin analogs. Their physical properties were compared with those of [11C]forskolin, and the biodistribution in mice was studied for [11C]forskolin and its analogs administered independently or with forskolin (10 μg).

MATERIALS AND METHODS


The deacetyl-precursors of [11C]forskolin and its analogs, i.e., 7-deacetylforskolin, 1,9-dideoxyforskolin and 7-deacetyl-1-deoxyforskolin, were synthesized from forskolin (Wako Pure Chemical Co. Ltd.), 1,9-dideoxyforskolin and 1-deoxyforskolin (Sigma Chemical Co. Ltd.), respectively, by the method of Pfeiffer (7). Those deacetyl-precursors were acetylated with [11C]acetylchloride in the presence of dimethylaminopyridine (Fig. 1). Five milligrams of deacetyl-precursors were dissolved in 0.5 ml of freshly distilled toluene, then 8 mg of dimethylaminopyridine was added. Thereafter [11C]acetylchloride, produced according to the procedures of Le Bars et al. (8), was introduced into the mixture at room temperature. The toluene was evaporated and the residue was dissolved in a small volume of acetonitrile:water = 1:1 and purified on a Shimadzu HPLC system (reversed-phase column; Megapak SIL-C18, 10 mm i.d. × 250 mm, Nihon Bunko Co. Ltd.; UV absorbance was monitored with a Shimadzu UV detector SPD-6AV) under elution with acetonitrile:water = 1:1, at the flow rate of 5 ml/min for [11C]forskolin and [11C]1-acetyl-7-deacetylforskolin, and 8 ml/min for [11C]1,9-dideoxyforskolin and [11C]1-deoxyforskolin. The retention times of [11C]forskolin, [11C]1-acetyl-7-deacetylforskolin, [11C]1,9-dideoxyforskolin and [11C]1-deoxyforskolin, were confirmed by authentic (forskolin, 6-acetyl-7-deacetylforskolin, 1,9-dideoxyforskolin and 1-deoxyforskolin;
Comparison of $^{14}$C-Forskolin and Its Analogs on the n-Octanol/Phosphate Buffer Partition Ratio

Synthesized $^{14}$C-forskolin, $^{14}$C-1-acetyl-7-deacetylforskolin, $^{14}$C-1,9-dideoxyforskolin and $^{14}$C-1-deoxyforskolin, (370 kBq each) were shaken for 30 sec with 2 ml of n-octanol and 2 ml of 0.1 M phosphate buffer (pH 7.4) in a tightly sealed tube. The mixtures were then incubated under shaking at 37°C for 30 min and centrifuged at 1000 × g for 5 min. The n-octanol/phosphate buffer partition ratio was calculated from the radioactivity between the n-octanol and phosphate buffer layer.

Biodistribution of $^{14}$C-Forskolin and Its Analogs in Mice

Groups of four mice were injected i.v. with 1.85 MBq of $^{14}$C-forskolin, $^{14}$C-1-acetyl-7-deacetylforskolin, $^{14}$C-1,9-dideoxyforskolin and $^{14}$C-1-deoxyforskolin. Five, ten and thirty minutes thereafter, the animals were killed and the radioactivity levels in various tissues were measured using a well-type scintillation counter (Pharmacia Co. Ltd.). In another experiment, mice were given either $^{14}$C-forskolin or $^{14}$C-1-acetyl-7-deacetylforskolin with 10 μg of forskolin and the levels in various tissues were examined.

RESULTS

Synthesis of $^{14}$C-Forskolin and Its Analogs

Preparative-HPLC profiles of synthesized $^{14}$C-forskolin, $^{14}$C-1-acetyl-7-deacetylforskolin, $^{14}$C-1,9-dideoxyforskolin and $^{14}$C-1-deoxyforskolin are shown in Figure 2. Carbon-11-forskolin and $^{14}$C-1-acetyl-7-deacetylforskolin
were eluted at 14.6 and 11.9 min, respectively. A smaller peak consisting of \(^{11}C\)6-acetyl-7-deacetylforskolin was observed at 8.7 min. The retention times of \(^{11}C\)forskolin, \(^{11}C\)1-acetyl-7-deacetylforskolin and \(^{11}C\)6-acetyl-7-deacetylforskolin, were confirmed by comparison with nonlabeled forskolin and 1-acetyl-7-deacetylforskolin synthesized from 7-deacetylforskolin and nonlabeled acetychloride, or nonlabeled authentic forskolin and 6-acetyl-7-deacetylforskolin. The retention times of \(^{11}C\)1,9-dideoxyforskolin and \(^{11}C\)1-deoxyforskolin were 18.0 and 18.5 min, respectively, which were in agreement with those of nonlabeled authentic 1,9-dideoxyforskolin and 1-deoxyforskolin. The synthesis of \(^{11}C\)forskolin and its analogs will be reported in detail later. The radiochemical yields of \(^{11}C\)forskolin, \(^{11}C\)1-acetyl-7-deacetylforskolin, \(^{11}C\)1,9-dideoxyforskolin and \(^{11}C\)1-deoxyforskolin, calculated from trapped \(^{11}C\)CO\(_2\) at the end of bombard, were 4.1%–5.7%, 8.2%–13.0%, 14.5%–15.2% and 17.5%–18.0%, respectively. The specific activities of purified \(^{11}C\)forskolin, \(^{11}C\)1-acetyl-7-deacetylforskolin, \(^{11}C\)1,9-dideoxyforskolin and \(^{11}C\)1-deoxyforskolin at the end of synthesis, were about 37–55.5 GBq/\(\mu\)mol. The radiochemical purity of \(^{11}C\)forskolin, \(^{11}C\)1-acetyl-7-deacetylforskolin, \(^{11}C\)1,9-dideoxyforskolin and \(^{11}C\)1-deoxyforskolin were 98%, 97%, 99% and 99%, respectively. The total amount of time required for the synthesis and purification of \(^{11}C\)forskolin and its analogs was about 35–45 min.

**Comparison of \(^{11}C\)Forskolin and Its Analogs on the n-Octanol/Phosphate Buffer Partition Ratio**

The n-octanol/phosphate buffer partition ratios of \(^{11}C\)forskolin, \(^{11}C\)1-acetyl-7-deacetylforskolin, \(^{11}C\)1,9-dideoxyforskolin and \(^{11}C\)1-deoxyforskolin were 56.9 ± 12.5, 66.8 ± 22.9, 377 ± 64 and 582 ± 270, respectively. Among these analogs, \(^{11}C\)1-acetyl-7-deacetylforskolin had the nearest n-octanol/phosphate buffer partition ratio to \(^{11}C\)forskolin. Carbon-11-1,9-dideoxyforskolin and \(^{11}C\)1-deoxyforskolin were more lipophilic than the \(^{11}C\)forskolin.

**Biodistribution of \(^{11}C\)Forskolin and Its Analogs in Mice**

Table 1 presents a comparison of \(^{11}C\)forskolin, \(^{11}C\)1-acetyl-7-deacetylforskolin, \(^{11}C\)1,9-dideoxyforskolin and \(^{11}C\)1-deoxyforskolin biodistribution in mice 30 min after injection. The biodistribution of \(^{11}C\)1,9-dideoxyforskolin and \(^{11}C\)1-deoxyforskolin differed from that of \(^{11}C\)forskolin and \(^{11}C\)1-acetyl-7-deacetylforskolin, in particular, in fat tissue and the brain. More \(^{11}C\)forskolin than \(^{11}C\)1-acetyl-7-deacetylforskolin accumulated in the heart and other organs. This tendency was significant in the heart. Loading of 10 \(\mu\)g of forskolin reduced the accumulation of \(^{11}C\)forskolin in the heart and other tissues, but accumulation did not significantly affect the \(^{11}C\)1-acetyl-7-deacetylforskolin accumulation in any tissue. Figure 3 shows the time-course of accumulation in the heart for \(^{11}C\)forskolin and \(^{11}C\)1-acetyl-7-deacetylforskolin administered alone or with 10 \(\mu\)g of forskolin. There was less \(^{11}C\)1-acetyl-7-deacetylforskolin in the heart than \(^{11}C\)forskolin and level of \(^{11}C\)forskolin radioactivity in the heart co-administered with forskolin was significantly lower at 30 min than at 5 or 10 min after injection.

**DISCUSSION**

We have previously reported the synthesis of \(^{11}C\)forskolin from 7-deacetylforskolin using \(^{11}C\)acetic acid and dicyclohexylcarbodiimide (3,4). Of the four OH groups on 7-deacetylforskolin, the 7-OH group was selectively acetylated by this method. However, because the DCC method comprises the process of obtaining \(^{11}C\)acetic acid free from water, it is not suitable for automation or remote operation. The present study showed the synthesis of \(^{11}C\)forskolin from 7-deacetylforskolin using \(^{11}C\)acetylchloride. Acetylation using \(^{11}C\)acetylchloride is suitable for automation or remote operation. However, this \(^{11}C\)-acetylchloride method produced nonselective acetylation of the four OH groups on 7-deacetylforskolin: \(^{11}C\)forsko-
TABLE 1  
Biodistribution of \([^{11}C]forskolin\) and Its Analogs 30 Minutes Postinjection  

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Liver</th>
<th>Spleen</th>
<th>Kidneys</th>
<th>Heart</th>
<th>Lungs</th>
<th>Fat</th>
<th>Muscle</th>
<th>Brain</th>
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<td>([^{11}C]forskolin)</td>
<td>0.230 ± 0.032</td>
<td>0.485 ± 0.036</td>
<td>0.310 ± 0.028</td>
<td>0.550 ± 0.038</td>
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<td>([^{11}C]forskolin + forskolin (10 \mu g))</td>
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Each value represents the mean ± s.d. for four mice.

FIGURE 3. Time-course of radioactivity in heart and serum for \([^{11}C]forskolin\) \((\mathbb{I})\), \([^{11}C]forskolin plus 10 \mu g of forskolin \((\mathbb{II})\), \([^{11}C]1\text{-acetyl-7-deacetylforskolin} \((\mathbb{III})\) and \([^{11}C]1\text{-acetyl-7-deacetylforskolin plus 10 \mu g of forskolin} \((\mathbb{III})\). Each value represents the mean ± s.d. of four mice. Asterisks signify values that are significantly \((p < 0.05, \text{Student's} \ t\text{-test})\) decreased from that of \([^{11}C]forskolin\) value.

Forskolin has high- and low-affinity sites for AC in human platelets and in the rat brain (6, 7). High-affinity sites for forskolin are associated with the Gsα and AC (9, 10). The specific binding of various forskolin analogs to Gsα and AC is consistent with their ability to activate AC (7). The 1- and 9-OH groups on the forskolin structure are critical for their ability to activate AC (5, 6). This led to our initial hypothesis that \([^{11}C]1\text{-acetyl-7-deacetylforskolin}, \ [^{11}C]1,9\text{-dideoxyforskolin} \text{and} \ [^{11}C]1\text{-deoxyforskolin} \text{might serve as \"nonspecific forskolin analogs\" that possess similar structures to that of forskolin.}

The results of the n-octanol/phosphate buffer partition ratio indicated that \([^{11}C]1\text{-acetyl-7-deacetylforskolin was\ldots}

Carbon-11-Forskolin • Sasaki et al. 1947
most similar to [11C]forskolin, but [11C]1,9-dideoxyforskolin or [11C]1-deoxyforskolin was different from [11C]forskolin. The biodistribution of [11C]1,9-dideoxyforskolin or [11C]1-deoxyforskolin was different from that of [11C]forskolin and [11C]1-acetyl-7-deacetylforskolin (Table 1), particularly in lipophilic tissues, such as fat and the brain, reflecting the n-octanol/phosphate buffer partition ratio. More [11C]forskolin accumulated in the heart, kidneys, liver and lungs than [11C]1-acetyl-7-deacetylforskolin. Moreover, loading of 10 μg of forskolin reduced the levels of [11C]forskolin in these tissues. This tendency was significant in the heart (Table 1 and Fig. 3). Rat myocardium has high affinity sites for forskolin with a Kd of 250 nM and a maximal binding capacity of 5 pmole/mg protein (6,7,9), respectively. However, significant specific-binding of [11C]forskolin was not observed in vivo in the brain (Table 1). This may be due to the low transfer constant of the [11C]forskolin from the blood circulation to the brain.

Forskolin has not only specific binding to Gsα and AC, but also nonspecific binding to the AC and membrane transport proteins (7,12-14), including the glucose transporter, nicotinic acetylcholine receptor, the gammaaminobutyric acid receptor, voltage-dependent K+ channels and possibly the P-glycoprotein multidrug transporter. However, adding inhibitors such as D-glucose, cytochalasin B and vinblastine, to the above membrane transport proteins, does not inhibit the high-affinity binding of [3H]forskolin to bovine brain membranes (7,12,14). These results indicate that Gsα and AC can be evaluated in vivo by using [11C]forskolin.

This study indicated that [11C]forskolin will be a useful imaging agent for the AC-related second messenger system. Moreover, [11C]1-acetyl-7-deacetylforskolin might be useful as a "nonspecific forskolin analog" to evaluate the proportion of nonspecific uptake in [11C]forskolin.

ACKNOWLEDGMENT

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REFERENCES