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

Toru Sasaki, Atsuko Enta, Tadashi Nozaki, Shin-ichi Ishii and Michio Senda

Positron Medical Center, Tokyo Metropolitan Institute of Gerontology, Itabashi, Tokyo; and Faculty of Hygienic Sciences, Kitasato University, Sagamihara, Kanagawa, Japan

To visualize the adenylate cyclase (AC)-related second messenger system, [^{11}C]forskolin, [^{11}C]1-acetyl-7-deacetylforskolin, [^{11}C]1,9-dideoxyforskolin and [^{11}C]1-deoxyforskolin were synthesized by acetylation of the respective deacetyl-precursors using [^{11}C]acetylchloride and dimethylaminopyridine. The radiochemical yield 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 were 5%, 10%, 15% and 18%, respectively. Since the 1- and 9-OH groups on the forskolin structure are critical for specific binding to AC (active type), we considered [^{11}C]1-acetyl-7-deacetylforskolin, [^{11}C]1,9-dideoxyforskolin and [^{11}C]1-deoxyforskolin to be nonspecific forskolin analogs. A comparative study of [^{11}C]forskolin and its analogs on the n-octanol/phosphate buffer (pH 7.4) partition ratio showed that [^{11}C]1-acetyl-7-deacetylforskolin has similar physical properties to [^{11}C]forskolin. In the mouse heart, kidneys, liver and lungs, more [^{11}C]forskolin accumulated than [^{11}C]1-acetyl-7-deacetylforskolin. Moreover, simultaneous [^{11}C]forskolin with forskolin (10 μg) administration reduced the accumulation of [^{11}C]forskolin particularly in the heart to the level of [^{11}C]1-acetyl-7-deacetylforskolin. These results indicate that [^{11}C]forskolin would be a useful imaging agent for the AC-related second messenger system.

J Nucl Med 1993; 34:1944–1948

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 [^{11}C]forskolin from 7-deacetylforskolin and [^{11}C]acetic acid using dicyclohexylcarbodiimide (DCC) (*3,4*). In this study, [^{11}C]forskolin, [^{11}C]1-acetyl-7-deacetylforskolin, [^{11}C]1,9-dideoxyforskolin and [^{11}C]1-deoxyforskolin were synthesized by acetylation of the respective deacetyl-precursors using [^{11}C]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 [^{11}C]1-acetyl-7-deacetylforskolin, [^{11}C]1,9-dideoxyforskolin and [^{11}C]1-deoxyforskolin to be nonspecific forskolin analogs. Their physical properties were compared with those of [^{11}C]forskolin, and the biodistribution in mice was studied for [^{11}C]forskolin and its analogs administered independently or with forskolin (10 μg).

MATERIALS AND METHODS

Synthesis of [^{11}C]Forskolin and Its Analogs

The deacetyl-precursors of [^{11}C]forskolin and its analogs, i.e., 7-deacetylforskolin, 7-deacetyl-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 Pfeuffer (*7*). Those deacetyl-precursors were acetylated with [^{11}C]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 [^{11}C]acetylchloride, produced according to the procedures of Le Bars et al. (*8*), was introduced into the mixture at room templeture. 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 Bunkko 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 [^{11}C]forskolin and [^{11}C]1-acetyl-7-deacetylforskolin, and 8 ml/min for [^{11}C]1,9-dideoxyforskolin and [^{11}C]1-deoxyforskolin. The retention times of [^{11}C]forskolin, [^{11}C]1-acetyl-7-deacetylforskolin, [^{11}C]6-acetyl-7-deacetylforskolin, [^{11}C]1,9-dideoxyforskolin and [^{11}C]1-deoxyforskolin, were confirmed by authentic (forskolin, 6-acetyl-7-deacetylforskolin, 1,9-dideoxyforskolin and 1-deoxyforskolin;

Received Apr. 15, 1993; revision accepted Jun. 23, 1993.

For correspondence or reprints contact: Dr. Toru Sasaki, Positron Medical Center, Tokyo Metropolitan Institute of Gerontology, 1-1 Naka-cho, Itabashi, Tokyo, 173, Japan.

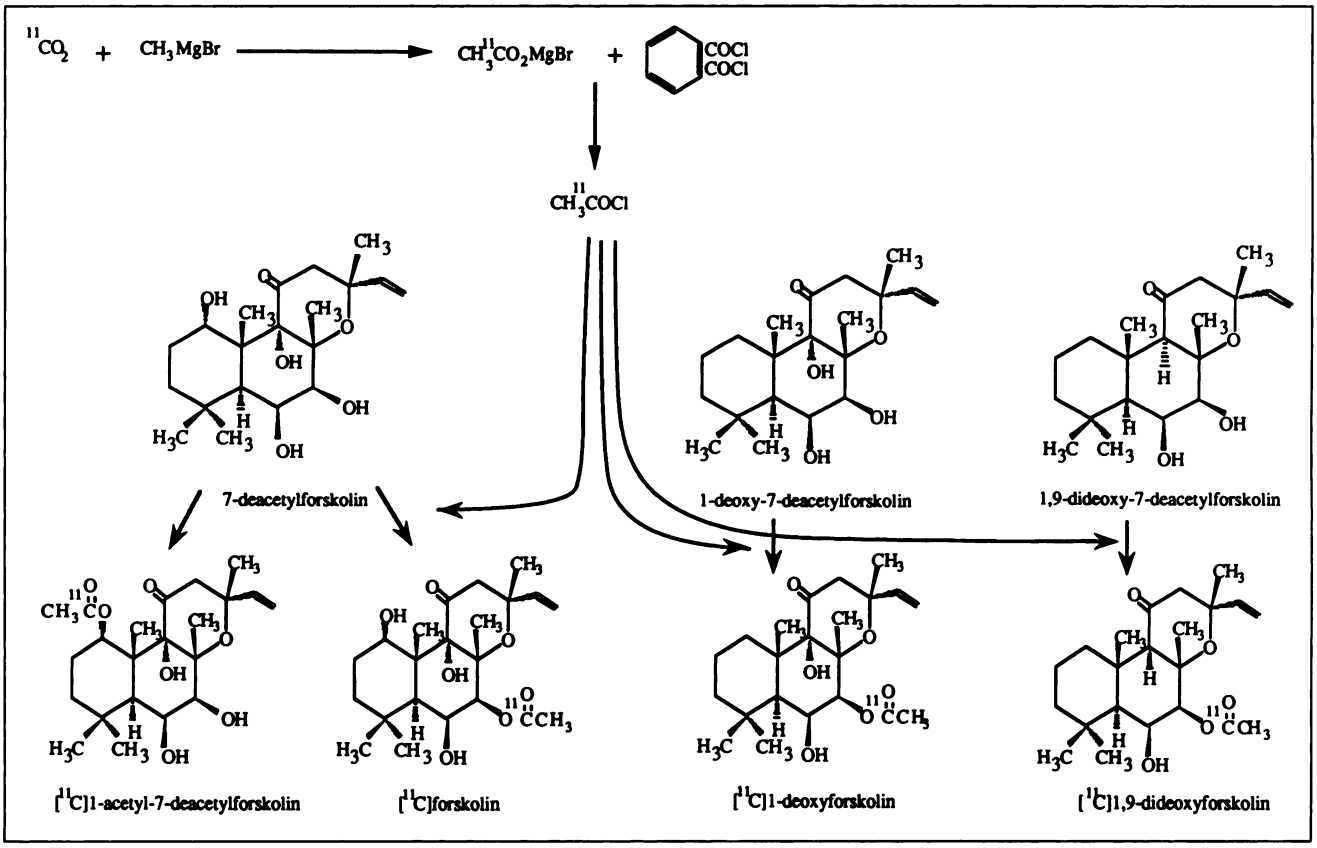

FIGURE 1. Synthesis scheme of [^{11}C]forskolin and its analogs.

Sigma Chemical Co. Ltd.) and synthesized standards (forskolin and 1-acetyl-7-deacetylforskolin). To determine the mass of [^{11}C]forskolin and its analogs, absorbance of UV detector (310 nm) in the analytical HPLC (reversed-phase column, Finepak SIL-C18S, 4.6 mm I.D. × 150 mm, Nihon Bunkko Co. Ltd., elution with acetonitrile:water = 1:1, at the flow rate of 1 ml/min) was pre-calibrated with corresponding mass of cold forskolin and its analogs. Specific activity of [^{11}C]forskolin and its analogs was calculated from radioactivity determined by a dose calibrator (Capintec) and mass. Nonradiolabeled forskolin and 1-acetyl-7-deacetylforskolin were synthesized in a similar manner as described above with 5 mg of deacetylforskolin, 9.7 μl of acetylchloride and 16.6 mg of dimethylaminopyridine. These products were identified by ^{1}H-NMR as follows. The ^{1}H NMR spectra were measured in solutions of $CDCl_3$ with a JEOL, GSX-400V spectrometer (Me_4Si as internal standard): Forskolin; ^{1}H NMR δ 1.04 (s, 3H, CH_3), 1.26 (s, 3H, CH_3), 1.35 (s, 3H, CH_3), 1.44 (s, 3H, CH_3), 1.71 (s, 3H, CH_3), 2.17 (s, 3H, acetyl), 2.20 (d, 1H, H-5), 2.47 (d, 1H, H-12), 3.09 (d, 1H, H-12), 4.46 (ddd, 1H, H-6), 4.58 (ddd, 1H, H-1), 4.99 (dd, 1H, vinylic-H), 5.30 (dd, 1H, vinylic-H), 5.48 (d, 1H, H-7), 5.94 (dd, 1H, vinylic-H). 1-Acetyl-7-deacetylforskolin; ^{1}H NMR δ 1.05 (s, 3H, CH_3), 1.28 (s, 3H, CH_3), 1.41 (s, 3H, CH_3), 1.49 (s, 3H, CH_3), 1.66 (s, 3H, CH_3), 2.03 (s, 3H, acetyl), 2.11 (d, 1H, H-5), 2.48 (d, 1H, H-12), 3.08 (d, 1H, H-12), 4.17 (d, 1H, H-7), 4.51 (ddd, 1H, H-6), 4.97 (dd, 1H, vinylic-H), 5.16 (ddd, 1H, H-1), 5.58 (ddd, 1H, H-1), 6.09 (dd, 1H, vinylic-H).

The [^{11}C]forskolin, [^{11}C]1-acetyl-7-deacetylforskolin, [^{11}C]1,9-dideoxyforskolin and [^{11}C]1-deoxyforskolin, obtained were evaporated to dryness and dissolved in 0.9% NaCl.

Comparison of [^{11}C]Forskolin and Its Analogs on the n-Octanol/Phosphate Buffer Partition Ratio

Synthesized [^{11}C]forskolin, [^{11}C]1-acetyl-7-deacetylforskolin, [^{11}C]1,9-dideoxyforskolin and [^{11}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 [^{11}C]Forskolin and Its Analogs in Mice

Groups of four mice were injected i.v. with 1.85 MBq of [^{11}C]forskolin, [^{11}C]1-acetyl-7-deacetylforskolin, [^{11}C]1,9-dideoxyforskolin and [^{11}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 [^{11}C]forskolin or [^{11}C]1-acetyl-7-deacetylforskolin with 10 μg of forskolin and the levels in various tissues were examined.

RESULTS

Synthesis of [^{11}C]Forskolin and Its Analogs

Preparative-HPLC profiles of synthesized [^{11}C]forskolin, [^{11}C]1-acetyl-7-deacetylforskolin, [^{11}C]1,9-dideoxyforskolin and [^{11}C]1-deoxyforskolin are shown in Figure 2. Carbon-11-forskolin and [^{11}C]1-acetyl-7-deacetylforskolin

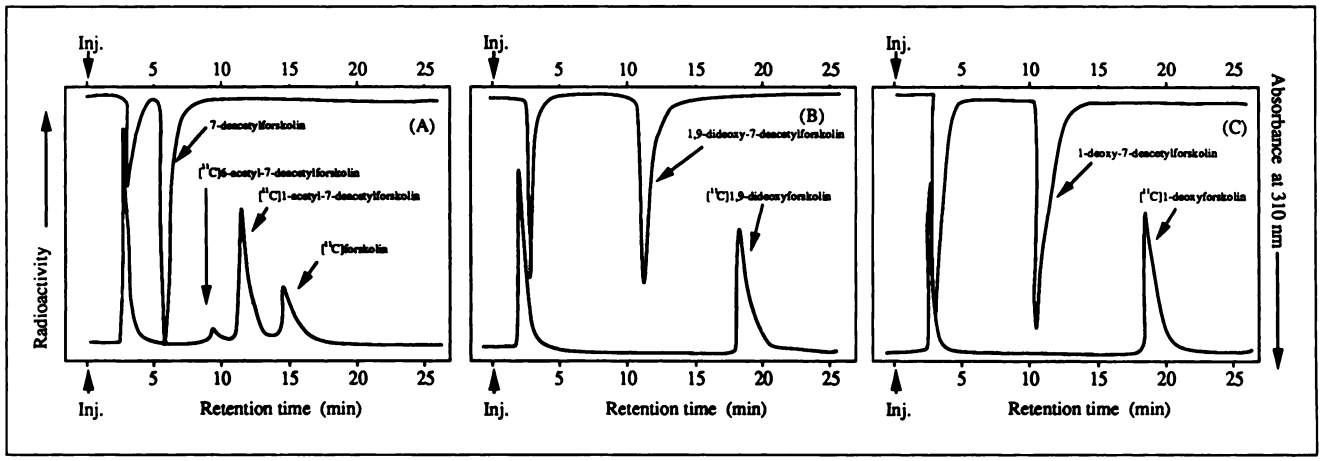

FIGURE 2. Purification of [^{11}C]forskolin, [^{11}C]1-acetyl-7-deacetylforskolin (A), [^{11}C]1,9-dideoxyforskolin (B) and [^{11}C]1-deoxyforskolin (C) using a preparative HPLC system.

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 acetylchloride, 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/μmol. 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 μg 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 μg 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

	%Dose/g tissue								
	Serum	Liver	Kidneys	Spleen	Heart	Lungs	Fat	Muscle	Brain
[^{11}C]forskolin	0.539 ± 0.083	6.82 ± 0.60	2.19 ± 0.35	0.642 ± 0.053	0.553 ± 0.056	0.604 ± 0.075	1.86 ± 0.21	0.476 ± 0.130	0.162 ± 0.026
[^{11}C]forskolin + forskolin (10 μg)	0.587 ± 0.047	5.89 ± 1.01	1.90 ± 0.46	0.486 ± 0.059	0.378 ± 0.045	0.468 ± 0.042	1.84 ± 0.21	0.336 ± 0.044	0.151 ± 0.019
[^{11}C]1-acetyl-7-deacetylforskolin	0.406 ± 0.142	4.24 ± 0.59	1.10 ± 0.12	0.511 ± 0.022	0.245 ± 0.017	0.395 ± 0.063	1.88 ± 0.49	0.375 ± 0.069	0.219 ± 0.034
[^{11}C]1-acetyl-7-deacetylforskolin + forskolin (10 μg)	0.385 ± 0.077	4.27 ± 0.38	1.09 ± 0.04	0.571 ± 0.077	0.260 ± 0.011	0.488 ± 0.061	1.60 ± 0.25	0.344 ± 0.004	0.174 ± 0.857
[^{11}C]1,9-dideoxyforskolin	0.212 ± 0.020	6.75 ± 1.21	1.83 ± 0.49	0.306 ± 0.020	0.809 ± 0.340	1.39 ± 0.44	5.04 ± 1.75	0.282 ± 0.025	0.236 ± 0.026
[^{11}C]1-deoxyforskolin	0.333 ± 0.048	8.84 ± 1.19	1.35 ± 0.29	0.436 ± 0.067	0.805 ± 0.153	0.804 ± 0.383	12.6 ± 3.8	0.308 ± 0.057	0.344 ± 0.037

Each value represents the mean ± s.d. for four mice.

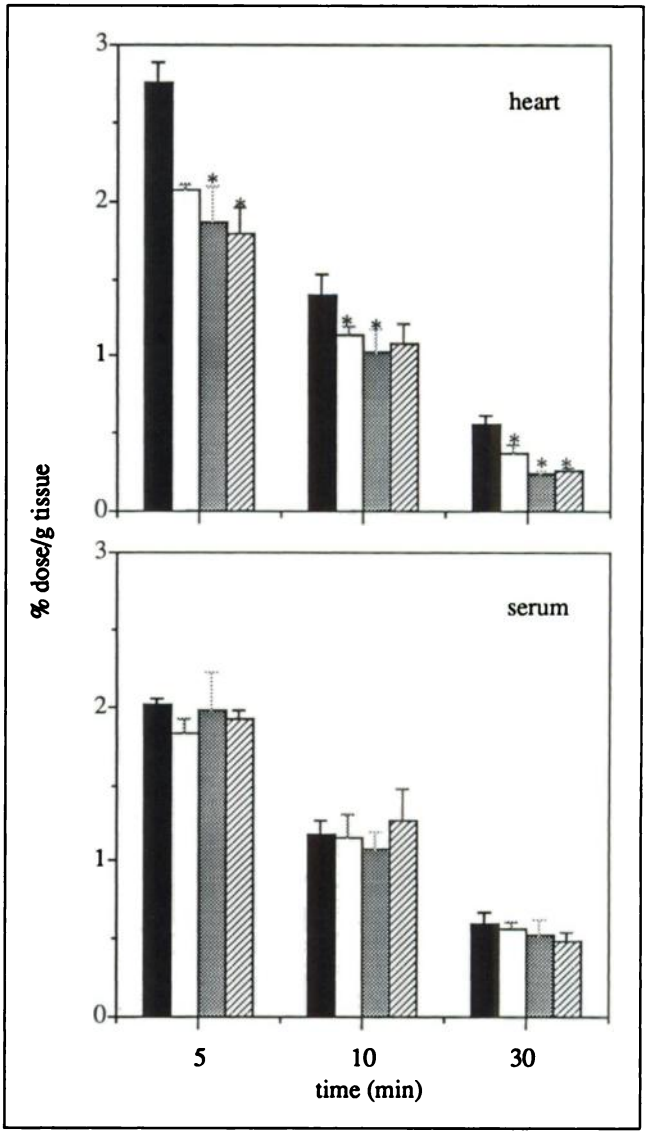

FIGURE 3. Time-course of radioactivity in heart and serum for [^{11}C]forskolin (■), [^{11}C]forskolin plus 10 μg of forskolin (□), [^{11}C]1-acetyl-7-deacetylforskolin (■) and [^{11}C]1-acetyl-7-deacetylforskolin plus 10 μg of forskolin (▨). Each value represents the mean ± s.d. of four mice. Asterisks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

lin, [^{11}C]1-acetyl-7-deacetylforskolin and [^{11}C]6-acetyl-7-deacetylforskolin (Fig. 2).

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-acetyl-7-deacetylforskolin, [^{11}C]1,9-dideoxyforskolin and [^{11}C]1-deoxyforskolin 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-acetyl-7-deacetylforskolin was

most similar to [^{11}C]forskolin, but [^{11}C]1,9-dideoxyforskolin or [^{11}C]1-deoxyforskolin was different from [^{11}C]forskolin. The biodistribution of [^{11}C]1,9-dideoxyforskolin or [^{11}C]1-deoxyforskolin was different from that of [^{11}C]forskolin and [^{11}C]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 [^{11}C]forskolin accumulated in the heart, kidneys, liver and lungs than [^{11}C]1-acetyl-7-deacetylforskolin. Moreover, loading of 10 μg of forskolin reduced the levels of [^{11}C]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 n*M* and a maximal binding capacity of 5 pmole/mg protein, as determined using [^{3}H]forskolin (*11*). These results indicate that [^{11}C]forskolin specifically binds to Gsα and AC in the heart and other tissues. Similarly, there are high affinity sites for forskolin in the brain, with a Kd and maximal binding capacity of about 10–40 n*M* and 40–900 fmole/mg protein (*6,7,9*), respectively. However, significant specific-binding of [^{11}C]forskolin was not observed in vivo in the brain (Table 1). This may be due to the low transfer constant of the [^{11}C]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 gamma aminobutyric 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 [^{3}H]forskolin to bovine brain membranes (*7,12,14*). These results indicate that Gsα and AC can be evaluated in vivo by using [^{11}C]forskolin.

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

ACKNOWLEDGMENT

This work was supported in part by a grant-in-aid from the Japanese government (no. 03770716).

REFERENCES

1. Robinson GA, Butcher RW, Sutherland EW. *Cyclic AMP*. Academic Press; 1971.
2. De Souza NJ, Dohadwalla AN, Forskolin RJ. A labdane diterpenoid with antihypertensive, positive inotropic, platelet aggregation inhibitory, and adenylate cyclase activating properties. *Medicinal Res Rev* 1983;3:201–219.
3. Sasaki T, Ogihara-Umeda I, Nishigori H, et al. Rapid synthesis of ^{14}C forskolin by a route suitable for ^{11}C labeling. *J Lab Compd Radiopharm* 1991;29:557–565.
4. Sasaki T, Yokota A, Ishiwata K, Senda M, Nozaki T, Nishigori H. Potentiality of C-11 forskolin for imaging of cAMP related second messenger system [Abstract]. *J Nucl Med* 1992;33:1025.
5. Santana CS, Guerrero JM, Reiter RJ. Effects of either forskolin, the 1,9-dideoxy derivative of forskolin, or 8-bromocyclic AMP on cyclic AMP and melatonin production in the Syrian hamster pineal gland in organic culture. *Neurosci Letters* 1989;103:338–342.
6. Seamon KB, Vaillancourt R, Edwards M, Daly JW. Binding of [^{3}H]forskolin to rat brain membranes. *Proc Natl Acad Sci USA* 1984;81:5081–5085.
7. Pfeuffer T. Synthesis of forskolin-agarose affinity matrices. *Methods Enzymol* 1991;195:44–65.
8. Le Bars D, Luthra SK, Pike VW, Lu Duc C. The preparation of a carbon-11 labelled neurohormone-[^{11}C]melatonin. *Appl Radiat Isot* 1987;38:1073–1077.
9. Nelson CA, Seamon KB. Binding of [^{3}H]forskolin to solubilized preparations of adenylate cyclase. *Life Sci* 1983;42:1375–1383.
10. Valverius P, Hoffman PL, Tabakoff B. Brain forskolin binding in mice dependent on and tolerant to ethanol. *Brain Res* 1989;503:38–43.
11. Schmidt K, Kukovetz WR. Evidence for forskolin binding sites in rat heart. *J Cyclic Nucleotide Protein Phosphorylation* 1985;10:425–438.
12. Joost HG, Habberfield AD, Simpson IA, Laurenza A, Seamon KB. Activation of adenylate cyclase and inhibition of glucose transport in rat adipocytes by forskolin analoges: structural determinations for distinct sites of action. *Molecular Pharmacol* 1988;33:449–453.
13. Morris DI, Robbins JD, Ruoho AE, Sutkowski EM, Seamon KB. Forskolin photoaffinity labels with specificity for adenylyl cyclase and the glucose transporter. *J Biol Chem* 1990;13377–13384.
14. Laurenza A, Robbins JD, Seamon KB. Interaction of aminoalkylcarbamates of forskolin with adenylyl cyclase: synthesis of an iodinated derivative of forskolin with high affinity for adenylyl cyclase. *Molecular Pharmacol* 1991;41:360–368.