No-Carrier-Added Carbon-11-Labeled *sn*-1,2- and *sn*-1,3-Diacylglycerols by [^{11}C]Propyl Ketene Method

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This article describes the preparation of *sn*-1,2-[^{11}C]diacylglycerols and *sn*-1,3-[^{11}C]diacylglycerols by a no-carrier-added reaction based on a labeling method using [1-^{11}C] propyl ketene, which is one of the most potent acylating agents. [1-^{11}C]Propyl ketene was produced by pyrolytic decomposition of [1-^{11}C]butyric acid and was trapped in pyridine containing L-α-palmitoyl-lysophosphatidylcholine, producing L-α-palmitoyl-2-[1-^{11}C]butyryl-*sn*-glycero-3-phosphorylcholine. We adopted an enzymatic reaction to remove the phosphorylcholine, in which L-α-palmitoyl-2-[1-^{11}C]butyryl-*sn*-glycero-3-phosphorylcholine was incubated with phospholipase C, hydrolyzing to produce 1-palmitoyl-*sn*-2-[1-^{11}C]butyrylglycerol. Total synthesis time was about 50 minutes and the specific activity was estimated at 93 GBq/μmol (2.5 Ci/μmol) at end of synthesis. Radiochemical yield was 3.8% based on the trapped $^{11}CO_2$. *sn*-1,3-[^{11}C]Diacylglycerol was also synthesized by [1-^{11}C]propyl ketene reaction with 1-palmitoyl-*sn*-glycerol in a single procedure. The regional brain tissue radioactivities obtained in *sn*-1,2-[^{11}C]diacylglycerol were higher than those of *sn*-1,3-[^{11}C]diacylglycerol, and the regional values varied widely. In autoradiography of brain slices from conscious rats, *sn*-1,2-[^{11}C]diacylglycerol incorporation sites were discretely localized, especially in the amygdala, cerebral cortex, and hippocampus, suggesting that intensive neuronal processing occurred in these areas on the basis of phosphatidylinositol turnover.

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Neuronal manifestations are driven by second messenger systems in synapses through the neuronal transmission process. Recently the receptor-mediated phosphatidylinositol (PI) response have been recognized as one of the most important systems in the CNS (*1,2*). If we could assess topographical receptor-mediated PI response by positron emission tomography (PET), these images would presumably reflect neuronal activation in particular neuronal pathways with a high sensitivity based on the common amplifying mechanism of the second messenger system. One method of assessing increases in PI turnover is based on a dual PI system consisting of inositol phosphates turnover and *sn*-1,2-diacylglycerol turnover. This has been used mainly in in vitro studies using a prelabeling method, in which there is an increased release of inositol phosphates from prelabeled lipids (*3*). However, these methods are not appropriate for PET studies because of limited tracer permeability in the blood–brain barrier and tracer degradation due to nonspecific phosphatases in blood. For these reasons, we focused on *sn*-1,2-diacylglycerol metabolism as one aspect of PI turnover and designed *sn*-1,2-[^{11}C] diacylglycerols (*sn*-1,2-[^{11}C]DAG) as an extrinsic tracer to become mixed into PI turnover and to be trapped intracellularly in vivo (*4*).

The synthesis method of *sn*-1,2-[^{11}C]DAG consists of two main processes: acylation to L-α-lysophosphatidylcholine, and enzymatic hydrolysis of phosphorylcholine moiety. The [1-^{11}C]propyl ketene method has been established (*5,6*); this labeling method is quite effective, especially for acylated carbohydrate compounds (*6–8*). We also discuss *sn*-1,3-[^{11}C]DAG and the potential of these new tracers for in vivo measurement of PI response by PET.

MATERIALS AND METHODS

Chemicals

L-α-Palmitoyl-lysophosphatidylcholine (1-palmitoyl-sn-glycero-3-phosphorylcholine), 1-palmitoyl-*sn*-glycerol, L-α-stearoyl-lysophosphatidylcholine (1-stearoyl-*sn*-glycero-3-phosphorylcholine), 1-stearoyl-*sn*-glycerol were the precursors, Phospholipase C type III from *Bacillus cereus* (Lecithinase C; EC 3.1.4.3), *n*-propyl bromide, lithium, and 4-dimethylaminopyridine (DMAP) were purchased from Wako Pure Chemicals Industries (Osaka). All diacylglycerols were analyzed by high-performance liquid chro-

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matography (HPLC) under the same conditions described later; ultraviolet (UV) spectra were measured in hexane in 1-cm cells in a sequential connected spectrophotometer (Shimadzu model SPD-6AV) using 242 nm in UV wavelength.

TLC Analysis

The *sn*-2 substitution of the L-α-acyl-lysophosphatidylcholine was verified by thin-layer chromatography (TLC) on layers of activated silica gel G developed in chloroform–methanol–ammonium hydroxide–water (60:30:8:2.3, v/v). The developed TLC was contacted with an imaging plate of Fuji Computed Radiography System (FCR), which was exposed for 1 hr and then calculated to obtain digitalized images.

Radio-HPLC Analysis

All diacylglycerols were analyzed by HPLC under the same conditions. Zorbax SIL (DuPont Instrument, 4.6 mm × 25 cm) was used for this study. HPLC was operated at room temperature, and diacylglycerols were separated by using hexane–ether–isopropyl alcohol (10:2:1 v/v). The flow rate was 1.8 ml/min. The radioactivity included in the eluent was sequentially counted using a multichannel analyzer.

[1-^{11}C]Propyl Ketene Synthesis: Ketene Method

The procedure described is essentially based on our previous report (*5*) though modified in respect to the purging process of [1-^{11}C]butyric acid (*6*). The scheme for the ketene generator is shown in Figure 1. The quartz glass column (ID: 7 mm, length: 240 mm) containing glass beads of 1mm in diameter was heated at 530°C, and the reaction vessel was connected. The gas mixture of 0.1% hydrogen chloride in helium (HCl/He); helium gasflow (He) for further dilution was through the P_2O_5 column for exclusion of the moisture from the gas mixture. C-11 labeled carbon dioxide was produced by a $^{14}N(p,\alpha)^{11}C$ reaction using a cyclotron (JSW: Baby Cyclotron system located in Nishijin Hospital); $^{11}CO_2$ gas was trapped in a reaction vessel immersed in liquid N_2. The supernatant (80 μl) of propyl lithium solution, prepared by the reaction of propyl bromide (3.3 mmol) with lithium (14.4 mmol) in dry diethyl ether (30 ml), was rapidly injected in a frozen mixture of $^{11}CO_2$ and nitrogen gas. Two min later, at room temperature, 100 μl of water was added, to decompose the lithium complex. After drying the mixture, dry hydrogen chloride in a carrier helium gas described as above was sprayed over the lithium salt of [1-^{11}C]butyric acid under a controlled gasflow. The conconcentration of hydrogen chloride in helium gas must be maintained at a low level within optimal conditions (1000 ppm, 6 ml/min). The reaction vessel was connected to the quartz glass tube and the [1-^{11}C]butyric acid vapor, purged by dry hydrogen chloride at room temperature, was transferred by a helium gasflow into the heated quartz glass tube at 530°C. [1-^{11}C]Propyl ketene generated by pyrolytic decomposition was trapped directly in the pyridine solution containing a precursor without the use of a cooling apparatus in order to preserve the reactivity. All the acylating reactions were performed under noncarrier-added conditions at room temperature and within 5 min.

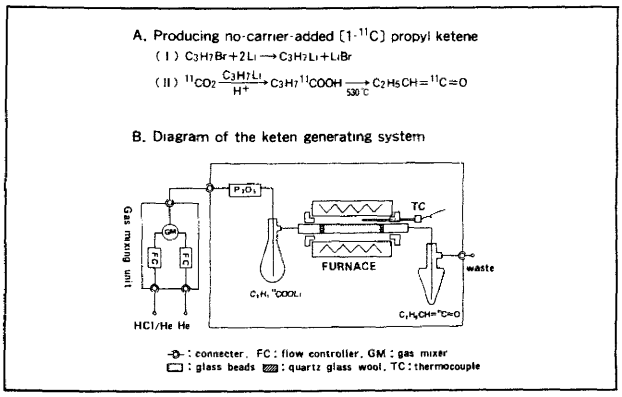

FIGURE 1. Producing procedures of NCA [1-^{11}C]propyl ketene and the generator system.

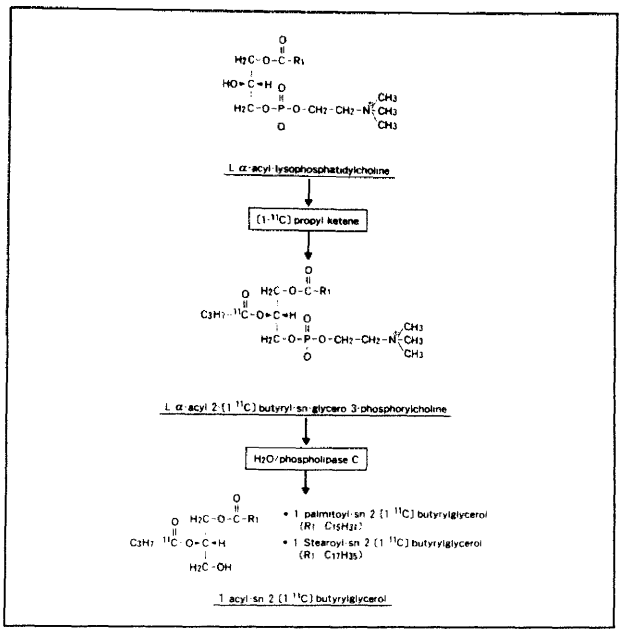

FIGURE 2. NCA preparation of sn-1,2-[^{11}C]DAG using PLC.

Synthesis of L-α-Palmitoyl-2-[1-^{11}C]Butyryl-*sn*-Glycero-3-Phosphorylcholine and Hydrolysis to 1-Palmitoyl-*sn*-2-[1-^{11}C]Butyrylglycerol by Using Phospholipase C

The scheme for the synthesis is shown in Figure 2. [1-^{11}C] Propyl ketene was induced to react in 300 μl of pyridine containing 1 μmol of L-α-palmitoyl-lysophosphatidylcholine and 0.5 μmol of DMAP at room temperature. Before the reaction, the mixture was suspended by sonification for 10 min. The acylating reaction was performed using [1-^{11}C]propyl ketene under no-carrier-added conditions at room temperature and within 5 min. From this reaction, L-α-palmitoyl-2-[1-^{11}C]butyryl-*sn*-glycero-3-phosphorylcholine was obtained as an [1-^{11}C]propyl ketene adduct of L-α-palmitoyl-lysophosphatidylcholine. Unreacted [1-^{11}C] propyl ketene and impurities were completely removed with pyridine by evaporation. TLC analysis was performed with radioactive L-α-palmitoyl-2-[1-^{11}C]butyryl-*sn*-glycero-3-phosphorylcholine. The R_f value was identified with unlabeled standard material. The residue containing L-α-palmitoyl-2-[1-^{11}C]butyryl-*sn*-glycero-3-phosphorylcholine was dissolved in 0.5 ml of potassium phosphate buffer (0.1 M, pH 7.0). To remove the phosphorylcholine by an enzymatic reaction in a two-phase system modified from that used by Zwaal et al. (*9*), 0.5 ml of the same buffer containing phospholipase C (PLC; 5 units, type III from *Bacillus cereus*) (*10*) and 1 ml of diethylether were added to the solution.

This hydrolysis procedure was performed for 10 min at 20°C, producing 1-palmitoyl-*sn*-2-[1-^{11}C]butyrylglycerol, which dissolved in the ether phase. At the end of the procedure, the *sn*-1,2-[^{11}C]DAG was further extracted from a water phase twice. *sn*-1,2-[^{11}C]DAG was verified by the radio-HPLC system. The same procedures were used in the synthesis of 1-stearoyl-*sn*-2-[1-^{11}C]butyrylglycerol via L-α-stearoyl-2-[1-^{11}C]butyryl-*sn*-glycero-3-phosphorylcholine using phospholipase C.

Synthesis of 1-Palmitoyl-*sn*-3-[1-^{11}C]Butyrylglycerol as a Nonspecific Diacylglycerol

[1-^{11}C]Propyl ketene was induced to react in 300 μl of pyridine containing 1 μmol of 1-palmitoyl-*sn*-glycerol and 0.5 μmol of DMAP at room temperature for 5 min. Then unreacted [1-^{11}C] propyl ketene and pyridine were completely removed using an evaporator. The same procedure was also used in the synthesis of 1-stearoyl-*sn*-3-[1-^{11}C]butyrylglycerol. The *sn*-1,3-DAG was verified by the HPLC system.

Biodistribution and In Vivo Autoradiography

Tracer distribution in rats (Male Wistar rats weighing 230–250 g) was measured by the following procedures. A dose of 0.8–2.5 mCi of [^{11}C]DAG, dissolved in 0.4 ml saline with 0.1% bovine serum albumin (BSA) and 0.5% dimethylsulfoxasol, was injected into each rat through the tail vein under anesthesia of 32 mg/kg of sodium pentobarbital. An intravenous bolus injection of each ^{11}C-labeled tracer was given, 30 min after intravenous (I.V.) injection; the rats were then killed by decapitation. The brain was rapidly removed and divided into several regions: amygdala, cerebral cortex, hippocampus, cerebellum, thalamus, basal ganglia, and pons. Tissue radioactivity was measured using a gamma counter and tissue samples were weighed. The uptake was expressed as (count/g tissue) × (1/total injected count) × 100%. The data obtained in triplicate experiments were presented as an average ±SD. In vivo autoradiography was performed under the same conditions described above. A rat was sacrificed 30 min after the injection of 3 mCi of 1-palmitoyl-*sn*-2-[1-^{11}C]butyrylglycerol. The brain was rapidly removed and frozen by powdered Dry Ice in preparation for autoradiography, which was reconstructed with the Fuji Computed Radiography system from adjacent sections of 40-μm thickness.

RESULTS

Synthesis of ^{11}C-Labeled *sn*-1,2-DAG

Propyl ketene is the most potent acylating agent (*5*), and this first reaction produced L-α-palmitoyl-2-[1-^{11}C] butyryl-*sn*-glycero-3-phosphorylcholine as a main product within 5 min. This reaction was verified by TLC with unlabeled standard material, which was synthesized by the authenticated procedure reported by Cabot and Jaken (*11*). The R_f value of main product, under a no-carrier-added condition, is 0.42 ± 0.01 (mean ±SD) in the radio-TLC system as described under Materials and Methods, a little larger than that of unlabeled standard material. However, these two spots showed a simultaneous 1:1 correspondence in the TLC plate. The radiochemical yield of L-α-palmitoyl-2-[1-^{11}C]butyryl-*sn*-glycero-3-phosphorylcholine calculated from the trapped $^{11}CO_2$ was approximately 25%. It is difficult to quantify a small amount of 1,2-diacylphosphatidylcholine by UV spectrophotometer. However, 12-deoxyphorbol 13-isobutyrate can be easily quantified in small amounts by UV spectrophotometry by reaction with [1-^{11}C]propyl ketene in the 20-hydroxyl group. The product, 12-deoxyphorbol 13-isobutyrate 20-[1-^{11}C]butyrate ([^{11}C]DPBB), can be employed as an indicator for estimating specific activity of the [1-^{11}C]propyl ketene. The specific activity of [1-^{11}C]propyl ketene was estimated to be 186–279 GBq/μmol (5–7.5 Ci/μmol). From these results, the specific activity of this [1-^{11}C]propyl ketene adduct was about 186 GBq/μmol (5 Ci/μmol) at end of synthesis. The second step of this procedure was an enzymatic hydrolysis using phospholipase C, which produced 1-palmitoyl-*sn*-2-[1-^{11}C]butyrylglycerol. This is the most important process because the radiochemical yield and purity of 1-palmitoyl-*sn*-2-[1-^{11}C]butyrylglycerol was influenced by the temperature of the enzymatic reaction related to the transformation to the 1,3-isomeric form. We performed this reaction at 20°C, as the most appropriate temperature. Under these conditions, the hydrolyzing ratio for the production of the 1-palmitoyl-*sn*-2-[1-^{11}C]butyrylglycerol per [1-^{11}C]propyl ketene adduct in this enzymatic reaction was about 15% of the radiochemical yield (total radiochemical yield, 3.8%, based on the trapped $^{11}CO_2$); and 96% purity was obtained. This procedure does not seem to be affected; however, under these conditions, 1,3-isomeric transformation did not occur. As shown in Figure 3, *sn*-1,2-[^{11}C]DAG was predominantly obtained at 20°C by the enzymatic reaction but was not obtained at 37°C, where a large contamination of the 1,3-isomeric form occurred. *sn*-1,2-[^{11}C]DAG was extracted from the water phase containing L-α-palmitoyl-lysophosphatidylcholine and unhydrolyzed L-α-palmitoyl-2-[1-^{11}C]butyryl-*sn*-glycero-3-phosphorylcholine, using diethyl ether. The specific activity was estimated at about 93 GBq/μmol (2.5 Ci/μmol) because of a time loss of about 20 min, including both PLC hydrolyzing (for 10 min) and ether extraction (for 10 min) procedures based on the value of L-α-palmi-

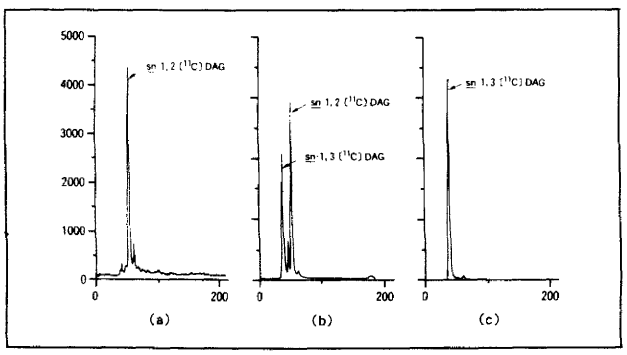

FIGURE 3. Radio-HPLC profiles of ^{11}C-labeled diacylglycerols. (A) *sn*-1,2-[^{11}C]DAG synthesized using PLC hydrolysis reaction at 20°C. (B) *sn*-1,2-[^{11}C]DAG synthesized using PLC hydrolysis reaction at 37°C. (C) *sn*-1,3-[^{11}C]DAG synthesized in a single procedure with [1-^{11}C]propyl ketene. × X-axis, a channel number of radio-HPLC (3 sec/channel). § Y-axis, each channel radioactivity of tracer.

toyl-2-[1-^{11}C]butyryl-*sn*-glycero-3-phosphorylcholine (186 GBq/μmol).

Synthesis of ^{11}C-Labeled *sn*-1,3-DAG

By contrast the synthetic procedure of *sn*-1,3-[^{11}C]DAG was very simple. The main procedure was an acylation to the 3-hydroxyl group of 1-palmitoyl-*sn*-glycerol by [1-^{11}C] propyl ketene reaction. When the reaction proceeded in a no-carrier-added condition, the reactivity of the 3-hydroxyl group was shown to be higher than that of the 2-hydroxyl group. The only C-11 adduct that it is possible to obtain is *sn*-1,3-[^{11}C]DAG in the no-carrier-added [1-^{11}C]propyl ketene reaction. The 3-hydroxylgroup of 1-palmitoyl-*sn*-glycerol is highly reactive with [1-^{11}C]propyl ketene. The radiochemical yield of 1-palmitoyl-*sn*-3-[1-^{11}C]butyrylglycerol, calculated from the trapped $^{11}CO_2$, was approximately 35%. As described above, the specific activity of this [1-^{11}C]propyl ketene adduct was greater than 186 GBq/μmol (>5 Ci/μmol), supported by the fact that the reactivity of the 3-hydroxyl group of 1-palmitoyl-*sn*-glycerol was clearly higher than that of the 2-hydroxyl group of L-α-palmitoyl-lysophosphatidylcholine. The radiochemical purity obtained using the radio-HPLC system as shown in Figure 3 was about 98%.

Regional Distribution of *sn*-1,2,- and *sn*-1,3-[^{11}C]DAG in Rat Brain

Regional distribution in the brain was investigated 30 min after injection of each tracer. As shown in Table 1, large differences between *sn*-1,2- and *sn*-1,3-[^{11}C]diacylglycerol values were seen in the amygdala, cerebral cortex, and hippocampus. These results suggest that the greater accumulation of *sn*-1,2-[^{11}C]DAG compared with that of *sn*-1,3-[^{11}C]DAG in a brain accurately reflects the phosphorylation process by 1,2-diacylglycerol kinase and that regional variations of the differences between *sn*-1,2-[^{11}C]DAG and *sn*-1,3-[^{11}C]DAG values can be attributed to regional biochemical events associated with PI turnover.

TABLE 1
Biodistribution of *sn*-1,2- and *sn*-1,3-[^{11}C]DAG

	1-Stearoyl 2-[1-^{11}C]butyryl glycerol (% dose/g)	1-Stearoyl 3-[1-^{11}C]butyryl glycerol (% dose/g)
Amygdala	0.41 ± 0.02*	0.26 ± 0.01
Cerebral cortex	0.37 ± 0.03*	0.25 ± 0.02
Hippocampus	0.34 ± 0.03†	0.23 ± 0.04
Cerebellum	0.34 ± 0.02 (ns)	0.29 ± 0.03
Thalamus	0.29 ± 0.02 (ns)	0.23 ± 0.04
Basal ganglia	0.26 ± 0.02‡	0.21 ± 0.02
Pons	0.25 ± 0.01 (ns)	0.21 ± 0.04

When compared with the values of *sn*-1,3-[^{11}C]DAG by t-test: *, $p < 0.01$; †, $p < 0.05$; ‡, $p < 0.1$, ns, nonsignificant.

Basal ganglia, including caudate nucleus and lentiform nucleus.

Biodistribution study was performed using male Wistar rats weighing 230–250 g. ^{11}C-labeled DAGs (0.8–2.5 mCi) were injected through a tail vein. The rats were killed at 30 min after injection, and the brain was divided into several tissues. The tissue radioactivity was measured using a gamma counter, and tissue samples were weighed. The regional uptake was expressed as (counts/g of tissue) × (1/total injected count) × 100%. Data present an average ±s.d. in triplicate experiments.

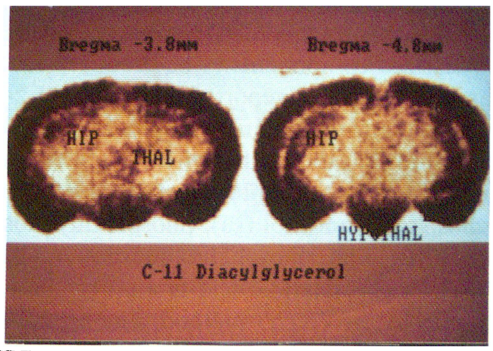

FIGURE 4. Typical autoradiography of 1-palmitoyl-sn-2-[1-^{11}C]-butyrylglycerol uptake sites in vivo in conscious rat brain. Brain slices were determined using a rat brain atlas (Paxinos G and Watson C, 1986). Brush-like pattern is demonstrated vertically to the cortical laminae. The amygdala (AMG), hippocampus (HIP), and hypothalamus (HYPOTHAL) can be demarcated. However, the thalamus (THAL) is unremarkable.

In Vivo Autoradiography in Rat Brain

In in vivo autoradiography of brain slices from conscious rats, *sn*-1,2-[^{11}C]DAG incorporation sites are discretely localized. Typical photography (Fig. 4) showed a demonstrable high optical density in the cerebral cortex, amygdala, hippocampus, and hypothalamus.

DISCUSSION

The efficiency required to obtain preferential brain images in vivo could be regulated by varying chemical properties in addition to the participation of the main biological processes. DAGs are all easily permeable through common biological membranes (*12,13*), and can readily pass through the blood–brain barrier. However, excessive lipophilicity is undesirable because of an increase in nonspecific tissue uptake of the tracers. Diacylglycerols, which have a short-chained substituent such as 2-butyryl residue, may be more suitable. The potency of 1-acyl-*sn*-2-butyrylglycerol is equivalent to that of dioleoylglycerol for the activation of protein kinase C (*11*), an the acyl chain need not be unsaturated as in the case of the monoenoic species because these DAG analogs could produce protein kinase C activation (*14*). The uptake mechanism could include exogenously added *sn*-1,2-DAG accumulation dependent on intracellular trapping and membrane trapping via phosphorylation by 1,2-diacylglycerol (DAG) kinase. This uptake mechanism may be the main factor involved in obtaining brain images, as the appearance of *sn*-1,2-DAG is transient, disappearing within a few seconds because of its conversion to PI turnover, prompted by membrane perturbation (*1*) via phosphatidic acid (*1,15*). A condition necessary for *sn*-1,2-[^{11}C]DAG to serve as a probe specific

to PI turnover is for phosphatidic acid to be produced by DAG kinase phosphorylation in the initial step, and for a series of polarized components to be produced for subsequent trapping. It has been reported that only *sn*-1,2-DAGs are used from the racemic mixture (*16*). In addition, our preliminary study has shown that *sn*-1,2-[^{11}C]DAG administered intravenously was rapidly metabolized to the ^{11}C-labeled phosphatidylinositol (PI), phosphatidylinositol 4-phosphate (PIP), and phosphatidylinositol 4,5-bisphosphate (PIP_2) via phosphatidic acid in a rat cerebral cortex within 20 min by the Folch's method. The regional tissue radioactivities obtained in *sn*-1,2-[^{11}C]DAG studies was higher than that of *sn*-1,3-[^{11}C]DAG and regional values varied widely. However, in *sn*-1,3-[^{11}C]DAG studies, the variation in tissue radioactivities was much lower (Table 1). On this account, *sn*-1,3-[^{11}C]DAG could be particularly suited for use in nonspecific uptake because the specificity of *sn*-1,3-[^{11}C]DAG for DAG kinase is extremely low (*17*). These ^{11}C-labeled diacylglycerols have the potential to be a new instrument for the visualization of PI response in the study of neurotransmission process.

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(continued from p. 1616)

SELF-STUDY TEST
Gastrointestinal Nuclear Medicine

ANSWERS

binding to persistent esophageal erosions secondary to the reflux esophagitis. Although it is true that free [^{99m}Tc]pertechnetate could be concentrated in ectopic gastric mucosa there is no thyroidal activity seen in the 60-min image to indicate that there is significant free pertechnetate present. Furthermore, Vasquez has found no significant absorption of free ^{99m}Tc from the gastrointestinal tract at 5 hours, when labeled sucralfate is adminstered orally. The retention of activity in the distal esophagus at 1 min could be due to decreased esophageal clearance. Karvelis has shown by radionuclide esophageal scintigraphy that decreased esophageal clearance is present in approximately 50% of patients with Barrett's esophagus. In studying these patients, therefore, it is helpful to perform an initial esophageal transit study prior to a labeled sucralfate study to ensure that any retention is not on the basis of delayed esophageal clearance. The normal mean $T_{1/2}$ for gastric emptying of sucralfate is 55 min. Hence, in normal subjects, it is common to see persistent ^{99m}Tc-sucralfate activity throughout the stomach. For this reason, it has been suggested that it is helpful to have patients "wash out" the stomach by drinking additional water approximately 30 min after administration of the sucralfate, particularly if one is attempting to identify gastric ulcerations. With gastritis there is an increase in the $T_{1/2}$ due to binding of sucralfate. However, because it is normal to have persistent sucralfate in the stomach at 90 min, one cannot conclude that gastritis is present in this patient.