Dual-Label Studies with [^{125}I]-3(R)/[^{131}I]-3(S)-BMIPP Show Similar Metabolism in Rat Tissues

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Biodistribution studies with the radioiodinated 3(R)- and 3(S)-isomers of 15-(p-iodophenyl)-3-methylpentadecanoic acid (BMIPP) in rats have shown that 3(R)-BMIPP has 20%–25% higher heart uptake than 3(S)-BMIPP (15–180 min). In contrast, the 3(S)-isomer has slightly higher liver uptake, and uptake in other tissues examined is similar. **Methods:** To evaluate the possible differences in metabolic fate of the two isomers, a mixture of [^{125}I]-3(R)/[^{131}I]-3(S)-BMIPP was administered to fasted female Fisher rats. Groups of rats (3 per group) were killed 15, 60 and 180 min after administration. Urine and feces were collected from a fourth group (n = 3) over 7 d. Samples of blood, heart, liver, lungs, kidney and urine were Folch extracted. The distributions of ^{125}I and ^{131}I in the organic (lipid), aqueous and pellet samples were determined. The lipid samples as well as the organic fractions from base-hydrolyzed triglyceride (TG) fractions and acid-hydrolyzed urine samples were then analyzed by thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC). **Results:** The relative distributions of ^{125}I and ^{131}I in the lipid, aqueous and pellet samples were similar for both isomers. Distribution of ^{125}I and ^{131}I in the various components of the lipid extracts observed by TLC (hexane:ether:HOAc, 70:30:1) was also similar, with principal incorporation into the free fatty acid (FFA) and TG pools. HPLC analyses (C_{18}) of the FFA fraction showed similar ^{125}I and ^{131}I profiles, corresponding to BMIPP, and the α-methyl-C_{14} (14-(p-iodophenyl)-3-(R,S)-methyltetradecanoic acid) and C_{12}, C_{10} and C_6 carbon chain-length catabolites. By TLC, radioactive components of ^{125}I and ^{131}I in the urine had the same TLC mobility as hippuric acid. HPLC analyses (C_{18}) of acid-hydrolyzed urine gave a single ^{125}I/^{131}I component with the same relative retention time as 2-(p-iodophenyl)acetic acid, which is the final α/β-oxidative BMIPP catabolite. Unexpectedly, HPLC of lipids from base-hydrolyzed TG from the heart tissue showed ^{125}I/^{131}I components with the same retention times as shorter-chain fatty acids, similar to the FFA fraction, with only low levels of activity detected in BMIPP. **Conclusion:** These results show that 3(R)-BMIPP and 3(S)-BMIPP are metabolized similarly in rat tissues and that higher myocardial extraction observed for 3(R)-BMIPP may reflect differences in the relative membrane transport of the two isomers.

Received Nov. 2, 1998; revision accepted Apr. 9, 1999.

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Key Words: radioiodinated fatty acids; 3(R)/3(S)-15-(p-iodophenyl)-3-methylpentadecanoic acid; cardiac imaging agents

J Nucl Med 1999; 40:1918–1927

Clinical SPECT comparison of regional myocardial distribution of the racemic [i.e., 3-(R,S)] ^{123}I-labeled 15-(p-iodophenyl)-3-methylpentadecanoic acid (BMIPP) fatty acid analog (Fig. 1) with flow tracers (sestamibi, ^{201}Tl) is an important method for identifying postischemic viable myocardium (*1–13*). The strategy for the original development of BMIPP was the expectation that introduction of the 3-methyl group would inhibit oxidative metabolism by the usual β-oxidative pathway (*1–5,14–16*). In this manner, minimal regional redistribution would occur during the relatively long periods for data acquisition that were required with single-head gamma cameras during the early 1980s. Initial planar imaging studies in humans showed prolonged myocardial retention of [^{123}I]-BMIPP in comparison with the [^{123}I]-15-(p-iodophenyl)pentadecanoic acid straight-chain analog (*13*). Using this unique metabolic trapping mechanism, high-quality SPECT images are obtained, representing the original "frozen" regional uptake pattern (*1–13*).

We have reported (*17,18*) resolution radioiodination and tissue distribution studies in rats with 3(R)-BMIPP and 3(S)-BMIPP (Fig. 1). These studies showed higher myocardial uptake of the 3(R)-BMIPP isomer in comparison with 3(S)-BMIPP. Whereas myocardial uptake of 3(R)-BMIPP is higher (20%–25%) than that observed for the 3(S)-isomer, 3(S)-BMIPP exhibits slightly higher liver uptake than 3(R)-BMIPP. The relative kinetics of myocardial washout for both isomers are similar, however, as well as the uptake and washout for other tissues examined (blood, kidneys, liver and lungs). A study in humans has demonstrated that there are no apparent differences in the myocardial uptake and regional distribution of ^{123}I 3(R)- and 3(S)-BMIPP (*19*).

To determine whether the differences observed in the rat studies resulted from differences in the relative metabolism of the two isomers, the present studies focused on a detailed evaluation of the relative uptake and flux of 3(R)-BMIPP and 3(S)-BMIPP through the various lipid pools in the heart

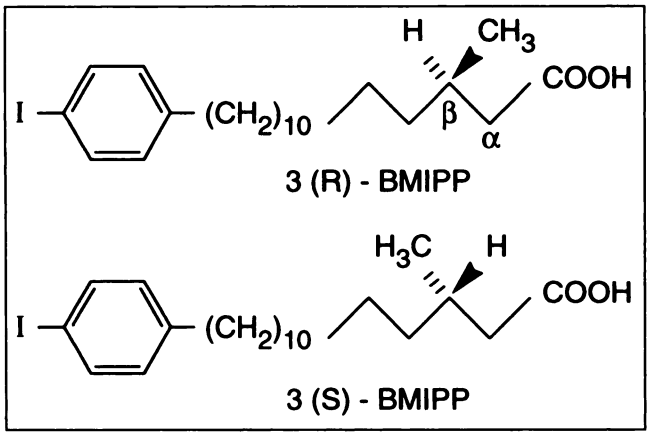

FIGURE 1. Structures of 15-(p-iodophenyl)-3-methylpentadecanoic acid (BMIPP) stereoisomers. The 3-methyl group present in BMIPP results in prolonged myocardial retention. ^{123}I-labeled racemic BMIPP, used as clinical agent, consists of equal mixture of 3(R)-BMIPP and 3(S)-BMIPP isomers.

and other representative tissues of rats. By administration of a dual-labeled [^{125}I]-3(R)-BMIPP/[^{131}I]-3(S)-BMIPP mixture to rats, each rat essentially served as its own control (*17,18*). A preliminary report of these studies was presented at the 1996 Annual Meeting of the European Association of Nuclear Medicine (*20*).

MATERIALS AND METHODS

All chemicals and solvents were analytical grade and were used without further purification. The 3(R)-15-phenyl-3-methylpentadecanoic acid (BMPPA) substrate was synthesized by a thiophene template approach described previously (*15–18*), which involved initial acylation of thiophene with the acid chloride prepared from the (R)-1-ethyl hydrogen 3-methylglutarate (designated as ethyl 3(R)-methyl glutarate; Fluka USA, Milwaukee, WI) and was >99% pure by gas-liquid chromatography (GLC) with an R/S ratio of >99:1 determined by high-performance liquid chromatography (HPLC) ($[\alpha]_D = -1.5°$, according to manufacturer; $-1.51°$, observed). The 3(S)-BMPPA substrate was obtained by acid hydrolysis of the chromatographically less polar diastereomeric amide obtained by separation of the amide mixture prepared by reaction of racemic BMIPP with (S)-(−)-α-methylbenzylamine (*17,18*). The amine was purchased from Aldrich Chemical Company (Milwaukee, WI) and was 99% enantiomeric excess determined by GLC ($[\alpha]_D = +39°$, according to manufacturer; +39°, observed). Authentic samples of 14-(p-iodophenyl)-3-(R,S)-methyltetradecanoic acid (AMIPT), 12-(p-iodophenyl)dodecanoic acid ($PIPC_{12}$), 6-(p-iodophenyl)hexanoic acid ($PIPC_6$) and 2-(p-iodophenyl)acetic acid (PIPA) were provided by Dr. Yoshihiro Yamamichi and colleagues from the Nihon Medi-Physics, Ltd., Central Research Laboratory (Tokyo, Japan). The sodium [^{131}I]iodide and [^{125}I]iodide were purchased from DuPont Biotechnology–New England Nuclear (Wilmington, DE).

Chromatography

Thin-layer chromatography (TLC) was performed using silica gel GF-254 coated on glass plates (Analtech Inc., Newark, DE) using 50% ether-hexane for amides and 6% methanol-chloroform for evaluation of free acids and other solvents where indicated. TLC analysis of radioactive samples was performed using 250 μm layers of silica gel coated on aluminum sheets (Merck Plates; Alltech Association, Inc., Deerfield, IL). For radiochemical analyses, the plates were marked into 10 equal sections and the sample applied. After development and drying, the plates were cut into sections, and the sections were counted in a gamma counter. Purification by absorption column chromatography was performed either on silica gel 200–400 mesh (Aldrich Chemical Co.) or silicic acid 60–200 mesh (Sigma Chemical Co., St. Louis, MO) with the solvents indicated. Analytical HPLC analyses were performed using 10 Partisil ODS-3 columns (Whatman, Inc., Clifton, NJ) with monitoring at 254 nm with an on-line variable wavelength detector (Waters Corp., Milford, MA) or a V^4 adsorbance detector (ISCO Inc., Lincoln, NB). Purification and analysis of radioiodinated compounds was performed using an on-line model 170 radioisotope detector (Beckman Coulter, Inc., Fullerton, CA) and ultraviolet detection at 254 nm or by collecting, at 1 min intervals, effluent samples, which were then counted in the gamma counter. The solvents and conditions for determination of radiochemical purity and analysis of lipid fractions are summarized in Table 1. For preparative purification of compounds, semipreparative HPLC columns were used with the same solvent systems indicated in Table 1 with ultraviolet monitoring at 254 nm.

Radioactivity Measurements

Tissue samples were counted in a Minaxi 5000 sodium iodide crystal gamma counter (Packard Instrument Co., Meriden, CT). An aliquot (2%–5%) of the injected dose was counted for calculation of the percentage injected dose per gram of tissue values. For dual-label studies, two windows were used for the 27- (^{125}I) and 364-keV (^{131}I) photopeaks. Corrections were made for the 17% spillover of ^{131}I in the 27-keV window. For determination of the injected dose and for determination of other absolute values, a calibrated HpGe solid-state detector (EG&G ORTEC, Oak Ridge, TN) coupled to a personal computer-based multichannel analyzer system (Nuclear Data/Canberra, Inc., Meriden, CT) was used. Data were analyzed using Accu-Spec spectroscopy software (Nuclear Data, Schaumburg, IL).

Preparation of Radioiodinated BMIPP Isomers

Radioiodinations were performed as described previously using the thallation-iodide displacement method (*15–18*). The BMPPA fatty acid substrate [i.e., 3(R)-BMPPA or 3(S)-BMPPA; 1.5 mg, 0.005 mmol] was combined with 5.4 mg (0.01 mmol) thallium trifluoroacetate in 2 mL trifluoroacetic acid, and the mixture was allowed to stand for 3–18 h at room temperature in the dark. The ^{131}I or ^{125}I solution in dilute base was combined with

TABLE 1
Conditions for HPLC Analyses Using Whatman ODS-3 Columns

Fraction analyzed	Solvent system
Free fatty fraction and acids released from basic hydrolysis of triglyceride fraction	Methanol:water:acetic acid, 90:10:0.01, 2 mL/min
Organic fraction from acid-hydrolyzed urine	Methanol:water:acetic acid, 50:50:0.01, 1.5 mL/min

HPLC = high-performance liquid chromatography.

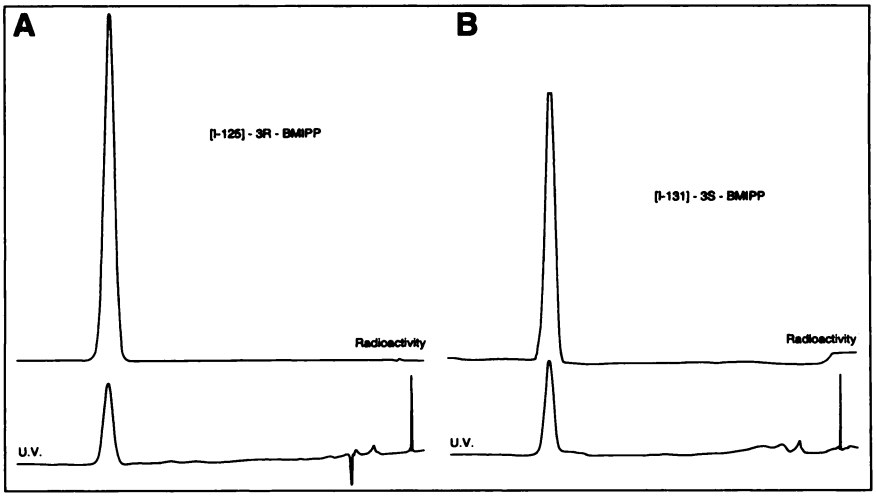

FIGURE 2. HPLC chromatogram of HPLC-purified [^{125}I]-3(R)-15-(p-iodophenyl)-3-methylpentadecanoic acid (BMIPP) (A) and [^{131}I]-3(S)-BMIPP (B). Ultraviolet (U.V.) traces are adsorbance at 254 nm.

0.5 mL of a standard aqueous KI solution (0.01 mmol, 1.6 mg/mL) and added to the substrate solution, which was heated at 95°C–100°C for 15 min in a heating block. After cooling to room temperature, the reaction mixture was poured into water (20–30 mL) and extracted with ethyl ether, and the organic layer was washed with an equal volume of dilute $NaHSO_3$, followed by several water washes to insure removal of the TFAA. The organic layer was then dried over Na_2SO_4, and the solvent was evaporated. Aliquots of the product were analyzed initially by comparison with an unlabeled BMIPP standard on TLC, using a SiO_2–gel flourescent indicator plate developed in 6% MeOH in $CHCl_3$ solution. After development and drying, the standard spot was visualized under ultraviolet light (254 nm), and the plate then was analyzed using a TLC radiochromatograph system. The major radioactive component in each case (>95%) corresponded with the standard. The [^{125}I]-3(R)-BMIPP and [^{131}I]-3(S)-BMIPP were purified by HPLC (Fig. 2) on a semipreparative C_{18} column using ethanol:isopropanol:water:acetic acid (80:5:15:0.1) as the solvent system. After purification, radiochemical purity was assessed with the same system. The BMIPP products were then stored as dry solids in the freezer until use.

Biodistribution Studies

Animal care and use procedures were in accordance with the guidelines for the care and use of laboratory animals of the National Institutes of Health and the Animal Welfare Act and were reviewed and approved by the National Laboratory Animal Care and Use Committee (Oak Ridge, TN). Each of the radioiodinated BMIPP isomers were dissolved separately in approximately 100 μL warm ethanol. The solutions then were slowly added separately, while stirring, to 5 mL of a solution of 6% bovine serum albumin, prepared in normal saline solution stirred at 40°C–45°C. The albumin mixtures were then each filtered through 0.22-μm filters (Millipore Corp., Bedford, MA), and aliquots were counted to determine the specific volume values. Appropriate aliquots of each solution were then combined and filtered through a 0.22-μm Millipore filter into a multidose vial, and an aliquot was counted to determine the injected dose values for each radioisotope. Samples of this solution were also used for counting with dissected tissues for calculations of injected dose values.

The rats were divided into four groups of three and fasted for 24 h before the experiment. After intravenous injection (0.5 mL) into a lateral tail vein of the methoxyfurane (Metophane; Mallinckrodt Veterinary, Inc., Mundelein, IL)-anesthetized rats, the animals were allowed food and water ad libitum. One group of rats was housed in metabolism cages, and urine and feces were collected daily. The other three groups were killed by cervical fracture after methoxyfurane anesthesia at 15, 60 and 180 min after the injection of the [^{125}I]-3(R)-BMIPP/[^{131}I]-3(S)-BMIPP mixture. The heart, liver, lungs and kidneys were removed, rinsed with saline, blotted dry

TABLE 2
Distribution of Radioactivity in Tissues of Groups of Fasted Female Fisher Rats (n = 3 per Group) After Intravenous Administration of Dual-Labeled Mixture of [^{125}I]-3(R)-BMIPP and [^{131}I]-3(S)-BMIPP*

Time after injection (min)	BMIPP isomer	Blood†	Heart†	Liver†	Lungs†	Thyroid†
15	[^{125}I]-3(R)-	2.20 ± 0.31	5.02 ± 0.21	2.79 ± 0.28	1.33 ± 0.29	17.16 ± 6.8
	[^{131}I]-3(S)-	2.13 ± 0.30	4.34 ± 0.34	3.93 ± 0.46	1.98 ± 0.10	15.58 ± 6.4
60	[^{125}I]-3(R)-	1.74 ± 0.12	3.17 ± 0.43	1.91 ± 0.18	1.29 ± 0.08	15.15 ± 7.6
	[^{131}I]-3(S)-	1.74 ± 0.11	2.32 ± 0.23	2.29 ± 0.26	1.53 ± 0.12	14.10 ± 7.2
180	[^{125}I]-3(R)-	1.60 ± 0.08	2.32 ± 0.69	1.22 ± 0.19	1.08 ± 0.05	10.26 ± 4.1
	[^{131}I]-3(S)-	1.61 ± 0.108	1.72 ± 0.45	1.10 ± 0.20	1.36 ± 0.13	8.53 ± 3.4

*Each rat was injected through a lateral tail vein with a mixture of 0.4 MBq (12 μCi) [^{125}I]-3(R)-BMIPP and 1.1 MBq (30 μCi) [^{131}I]-(3S)-BMIPP complexed to a 6% bovine serum albumin solution.
†Values given in percentage injected dose per gram of tissue ± SD.
BMIPP = 15-(p-iodophenyl)-3-methylpentadecanoic acid.

TABLE 3

Mean Percentage Distribution of ^{125}I and ^{131}I in Organic, Aqueous and Pellet Fractions from Folch-Extracted Rat Tissues After Intravenous Administration of Mixture of [^{125}I]-3(R)-BMIPP and [^{131}I]-3(S)-BMIPP (n = 3 per Group) to Fasted Female Fisher Rats

Tissue	Time after injection (min)	Organic phase		Aqueous		Pellet	
		^{125}I	^{131}I	^{125}I	^{131}I	^{125}I	^{131}I
Heart*	15	79.5 ± 2.90	79.6 ± 5.12	4.98 ± 0.76	5.87 ± 0.93	16.5 ± 2.61	14.5 ± 1.73
	60	75.1 ± 14.1	75.7 ± 2.06	5.48 ± 1.39	7.68 ± 2.06	19.5 ± 6.50	17.1 ± 4.54
	180	79.0 ± 18.6	80.0 ± 18.4	3.38 ± 1.56	4.45 ± 2.09	17.6 ± 5.73	15.5 ± 4.23
Blood*	15	—	—	41.1 ± 0.61	40.6 ± 2.07	44.4 ± 30.7	43.7 ± 31.3
	60	11.6 ± 0.94	12.1 ± 1.00	38.5 ± 3.33	40.2 ± 3.85	48.8 ± 21.4	47.7 ± 21.9
	180	5.1 ± 1.97	5.5 ± 2.03	24.8 ± 7.57	26.5 ± 8.37	70.1 ± 8.34	68.0 ± 11.6
Liver*	15	44.9 ± 7.01	45.9 ± 5.66	15.3 ± 3.02	15.3 ± 2.86	39.9 ± 8.10	38.8 ± 8.18
	60	38.5 ± 6.57	38.7 ± 5.48	15.6 ± 1.15	15.6 ± 0.86	45.5 ± 7.21	45.7 ± 6.60
	180	37.8 ± 10.9	37.8 ± 9.90	17.7 ± 1.26	17.7 ± 1.24	45.3 ± 12.9	44.4 ± 10.9
Lung*	15	44.9 ± 5.68	48.8 ± 5.25	31.1 ± 4.31	30.2 ± 4.05	23.9 ± 5.21	21.1 ± 4.32
	60	35.9 ± 3.08	37.4 ± 2.39	37.7 ± 5.13	37.4 ± 4.59	26.7 ± 4.96	25.2 ± 6.76
	180	40.7 ± 9.21	37.3 ± 3.56	32.9 ± 5.85	35.0 ± 6.46	26.4 ± 9.72	27.7 ± 9.06
Kidney*	15	29.2 ± 0.66	31.2 ± 0.71	42.5 ± 3.38	41.8 ± 4.59	28.3 ± 12.3	26.9 ± 11.9
	60	33.1 ± 0.91	32.4 ± 2.86	16.9 ± 1.68	18.8 ± 1.10	19.9 ± 1.04	18.9 ± 2.20
	180	24.2 ± 3.17	23.8 ± 2.83	55.3 ± 5.31	56.1 ± 4.78	20.8 ± 2.40	20.1 ± 1.41

*Values given in percentage injected dose per gram of tissue ± SD.
BMIPP = 15-(p-iodophenyl)-3-methylpentadecanoic acid.

and weighed in tared vials. Heparinized blood samples were obtained from the chest cavity after removal of the heart. A section of the trachea containing the thyroid gland was removed, and the weight was calculated as 0.7% of the animal weight. Samples were counted in a Packard Minaxi 5000 auto gamma counter.

Lipid Extraction and Analysis

Tissue samples were extracted by the traditional Folch technique as described earlier (*21–23*), using a loose-fitting Potter-Elmejahn ground-glass homogenizer (Kontes, Vineland, NJ). For the heart, kidneys and lungs, the chilled samples were finely minced in a watch glass and then homogenized for 3 min in 10 mL 2:1 chloroform:methanol mixture. Because of the large tissue mass, livers were weighed, and then 0.5- to 1-g aliquots were taken, minced and extracted in the same manner. Blood samples were homogenized directly. The extracts were filtered through filter paper into centrifuge tubes, and the filters were washed with an additional 1 mL chloroform. The filtrates were thoroughly mixed after

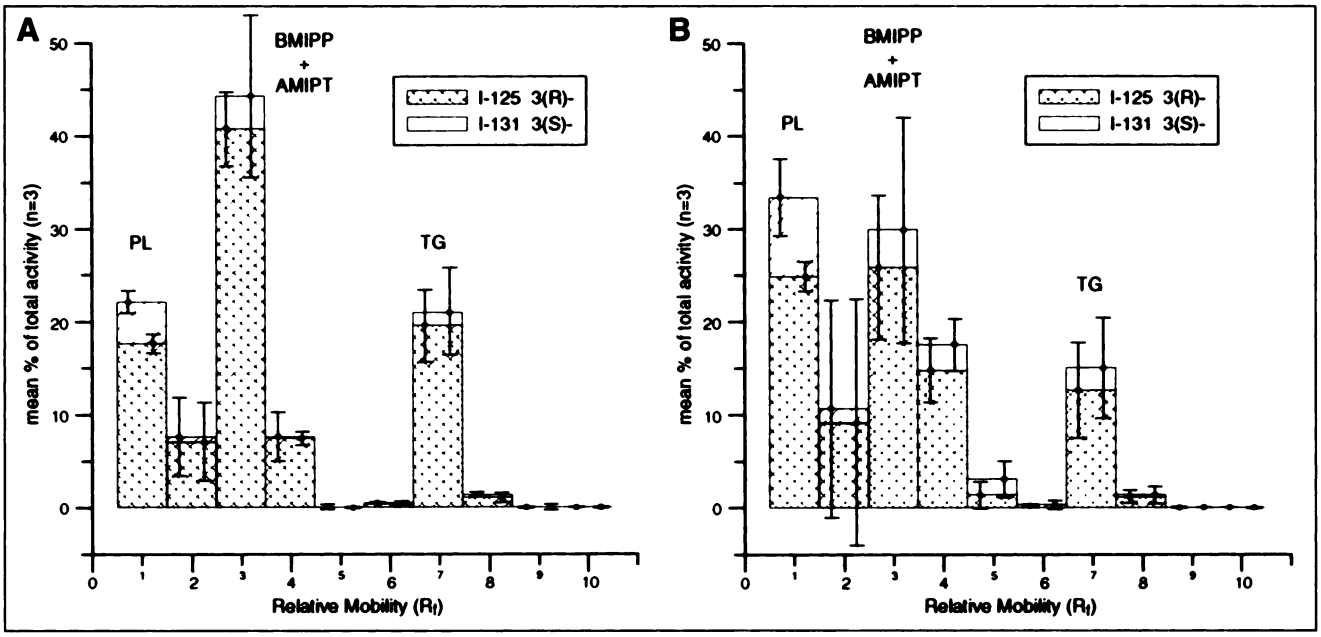

FIGURE 3. Distribution of ^{125}I and ^{131}I in heart lipids after 60 (B) and 180 (A) min. TLC system = petroleum ether:ethyl ether:acetic acid, 70:30:1. AMIPT = 14-(p-iodophenyl)-3-(R,S)-methyltetradecanoic acid; BMIPP = 15-(p-iodophenyl)-3-methylpentadecanoic acid.

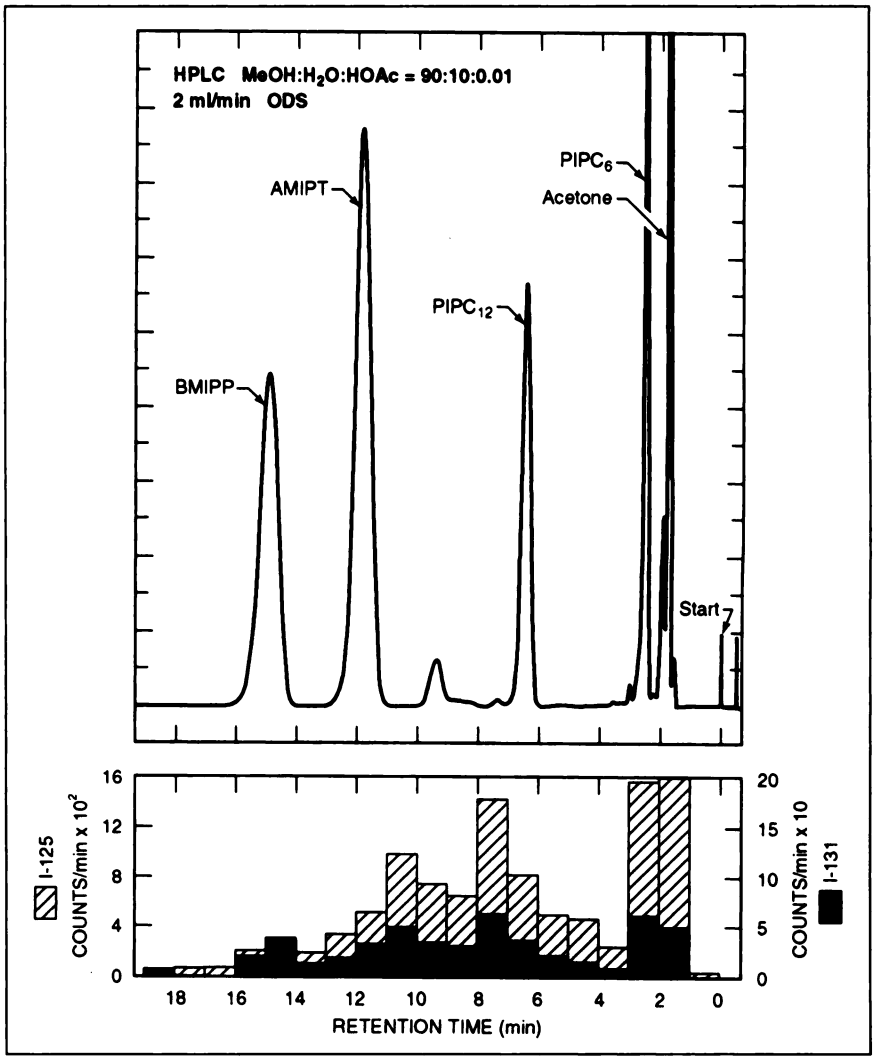

FIGURE 4. High-performance liquid chromatography (HPLC) of fatty acid fraction obtained by basic hydrolysis of pooled triglyceride fraction purified by column chromatography of 30-min heart lipids. Standards: 14-(p-iodophenyl)-3-(R,S)-methyltetradecanoic acid (AMIPT), 12-(p-iodophenyl)dodecanoic acid ($PIPC_{12}$), 6-(p-iodophenyl) hexanoic acid ($PIPC_6$) and PIPA. Data represent mean values from two analyses each, and error bars are therefore not shown (Table 1 shows conditions). BMIPP = 15-(p-iodophenyl)-3-methylpentadecanoic acid.

addition of 2 mL normal saline and centrifuged at low speed for 5–10 min to separate the organic (lower) and aqueous layers. The organic layers were removed carefully with a pipette and filtered through a short column of anhydrous sodium sulfate and evaporated to dryness under an argon stream. The evaporated organic and aqueous fractions and the filter paper containing the tissue pellet were then counted in a gamma counter. The organic residues were analyzed by TLC using silica gel–coated aluminum sheets and the following solvent systems: system A, petroleum ether:ethyl ether: acetic acid (70:30:1); system B, 15% methanol-chloroform; system C, 30% methanol-chloroform; and system D, chloroform:acetic acid (2:1). The sheets were dried after development, cut into 10 segments and counted.

Urine and Fecal Analyses

Urine and feces were collected daily for 7 d. Most of the activity was excreted in the first 2 d, so samples of the urine from day 1 and day 2 were applied to silica gel plates and analyzed in the three solvent systems indicated above. Urine samples were also hydrolyzed with hydrochloric acid. Concentrated HCl (2 mL) was cautiously added to aliquots of the first-day urine (1 mL) collected from rats housed in metabolism cages. The mixture was heated in a Teflon (DuPont, Wilmington, DE)-lined steel bomb for 2 h at 165°C–175°C. After cooling, the mixture was carefully poured into water, and the aqueous mixture was then extracted with ether as described previously (*17,18*).

Hydrolysis of Radioiodinated Metabolites

For basic hydrolysis of the triglyceride (TG) fractions, the combined lipids extracted from hearts obtained from rats 30 min after injection were pooled and chromatographed on a silicic acid column (1.5 cm internal diameter × 20 cm length) by elution with chloroform. The initial radioactive component (fractions 6–8) consisted of the nonpolar TG fraction, which was confirmed by TLC. The column-purified TG fraction was then dissolved in ethanol (4–5 mL), and, after the addition of 1 N NaOH solution (1 mL), the mixture was refluxed for 30 min, cooled, diluted with water (10 mL) and extracted with ethyl ether. The ether fraction was washed with water (2 × 20 mL) and dried over sodium sulfate, and the solvent was removed in vacuo to provide the nonsaponifiable (FFA) fraction, which was then analyzed by HPLC.

RESULTS

The distributions of ^{125}I [from 3(R)-BMIPP] and ^{131}I [from 3(S)-BMIPP] (Table 2) were similar to the results of experiments reported earlier (*17,18*). The results of the Folch extraction studies (Table 3) showed essentially no

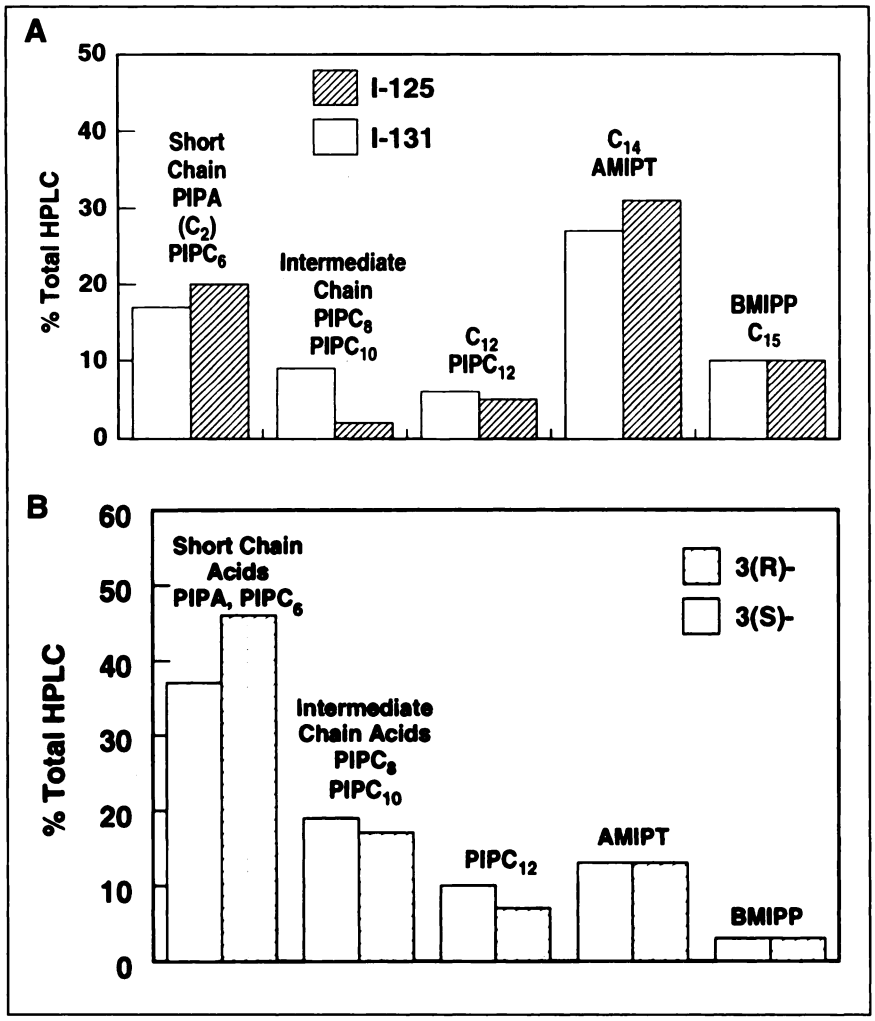

FIGURE 5. Summary of high-performance liquid chromatography (HPLC) data of FFA fractions from myocardial lipids obtained from animals killed after 60 min (A) and after 180 min (B). Standards: 14-(p-iodophenyl)-3-(R,S)-methyltetradecanoic acid (AMIPT), 12-(p-iodophenyl)dodecanoic acid ($PIPC_{12}$), 6-(p-iodophenyl)hexanoic acid ($PIPC_6$) and 2-(p-iodophenyl)acetic acid (PIPA). Data represent mean values from two analyses each, and error bars are therefore not shown (Table 1 shows conditions). BMIPP = 15-(p-iodophenyl)-3-methylpentadecanoic acid; $PIPC_8$ = 8-(p-iodophenyl)octanoic acid; $PIPC_{10}$ = 10-(p-iodophenyl)decanoic acid.

differences (P = 0.05) in the distribution of [^{125}I]-3(R)-BMIPP and [^{131}I]-3(S)-BMIPP in the organic, aqueous and pellet fractions and were similar to data reported earlier for the metabolism of racemic BMIPP in rats in vivo (*21–23*), Langendorff-perfused rat hearts (*24–27*) and dogs in vivo (*28,29*). Radioactivity migrating in the region of the free fatty acids (FFAs) by TLC in our earlier studies was identified as unmetabolized BMIPP (*21–23,25,26*). However, catabolism of BMIPP by a process involving initial α-oxidation preceding subsequent β-oxidation has been experimentally confirmed (*30*). The AMIPT metabolite has been isolated and identified, as we had originally suggested (*1–6,13*).

The availability of authentic samples of AMIPT, $PIPC_{12}$, $PIPC_6$ and PIPA provided an opportunity in these studies to compare the TLC and HPLC mobility of the radioiodinated metabolites formed from 3(S)-BMIPP and 3(R)-BMIPP. By comparing the relative mobility of the radioactive metabolites with the authentic standards (Fig. 3), results from the present HPLC study confirm that the radioactive catabolites from both [^{125}I]-3(R)-BMIPP and [^{131}I]-3(S)-BMIPP correspond to the C_{14} chain-length AMIPT metabolite and other short-chain metabolites.

In addition to the evaluation of the identity of components of the FFA pool in tissue extracts after intravenous administration of the radioactive dual-labeled 3(S)/3(R)-BMIPP mixture, we also evaluated the acidic components obtained by saponification of the column-purified TG fraction obtained from the myocardial lipid extracts. The nonsaponifiable fraction was analyzed by both TLC and HPLC analyses. Whereas TLC showed the presence of components more polar than BMIPP and AMIPT, which suggested short-chain acidic metabolites, HPLC analysis (Fig. 4) confirmed that the acidic fraction released by basic hydrolysis appears to consist primarily of short-chain metabolites rather than BMIPP, as had been presumed initially (*1–4*). In earlier studies, only the TG fraction was isolated and not hydrolyzed. Analysis of the radioactive lipids extracted from blood and liver also showed very similar distribution patterns for the two radioisotopes by TLC. Essentially identical relative distribution patterns for both radioisotopes were also found by HPLC analysis of the heart lipids (Fig. 5) and for the lipids released by hydrolysis of the radioactive TGs isolated from the pooled lipids 30 min after injection (Fig. 4).

Urine and feces, collected over a 1-wk period, indicated rapid excretion of radioactivity (Fig. 6). The relative radioac-

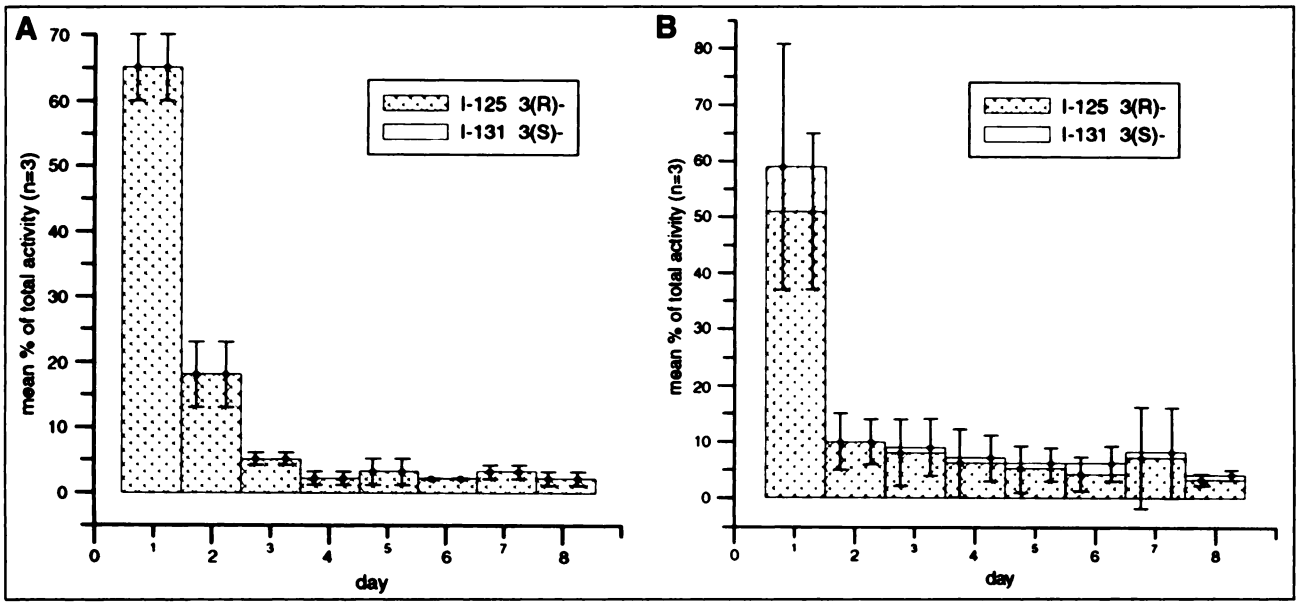

FIGURE 6. Excretion of radioactivity in urine (A) and feces (B) from rats housed in metabolism cages (n = 3). Mean % values are for total urine and feces, respectively, excreted over period indicated.

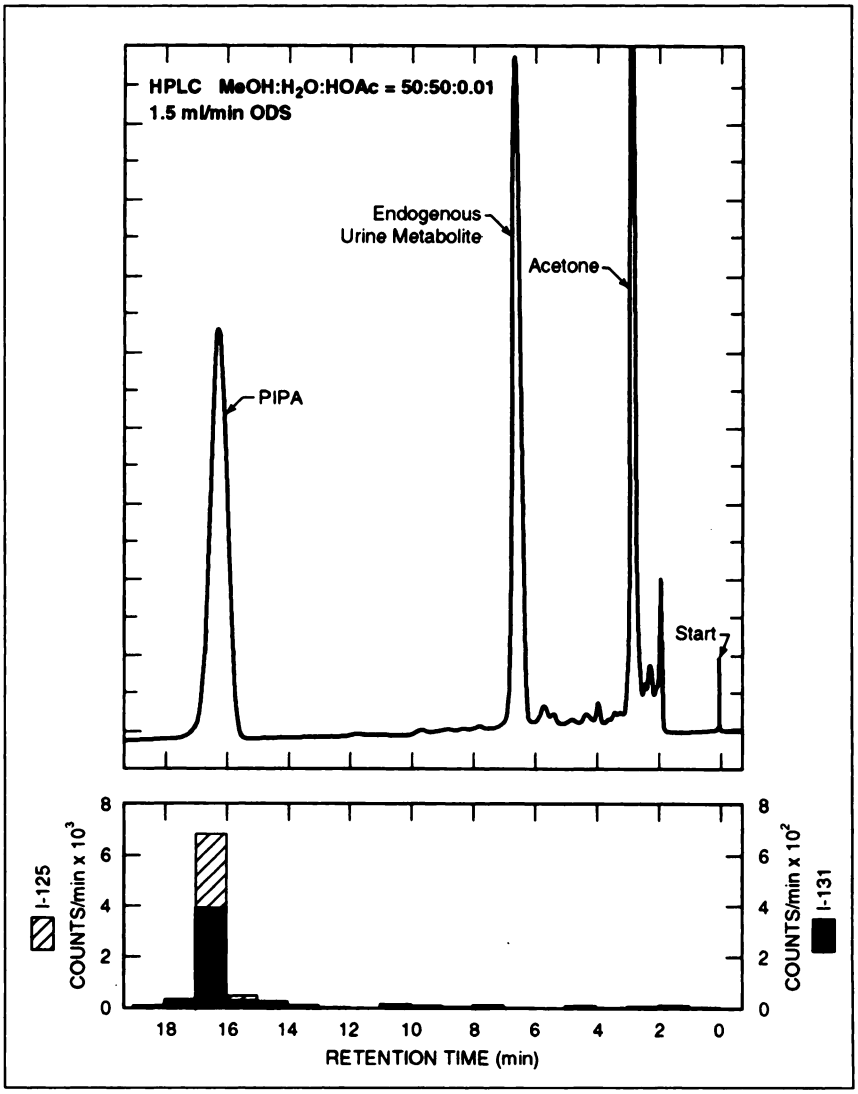

FIGURE 7. High-performance liquid chromatography (HPLC) of nonsaponifiable fraction obtained by acid hydrolysis of urine obtained over 24-h period.

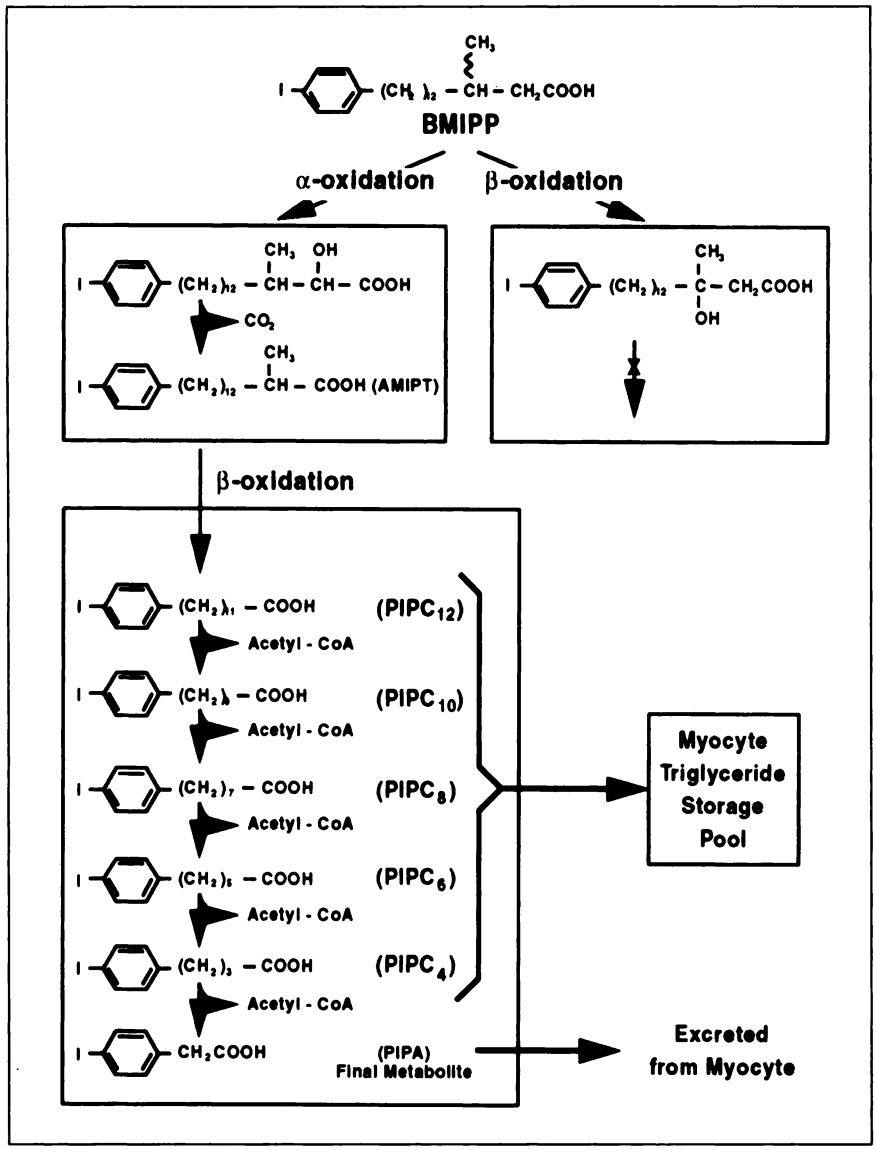

FIGURE 8. Proposed scheme for metabolism of 15-(p-iodophenyl)-3-methylpentadecanoic acid (BMIPP) in rat tissues. AMIPT = 14-(p-iodphenyl)-3-(R,S)-methyltetradecanoic acid; PIPA = 2-(i-iodophenyl)acetic acid; $PIPC_4$ = 4-(p-iodophenyl)butyric acid; $PIPC_6$ = 6-(p-iodophenyl)hexanoic acid; $PIPC_8$ = 8-(p-iodophenyl)octanoic acid; $PIPC_{10}$ = 10-(p-iodophenyl)decanoic acid; $PIPC_{12}$ = 12-(p-iodophenyl)dodecanoic acid.

tivities excreted from 3(R)-BMIPP (^{125}I) and 3(S)-BMIPP (^{131}I) were very similar. Analysis of the radioactive components of the urine indicated the presence of a single component that showed the same retention times as hippuric acid, suggesting that the metabolites were conjugated to an amine similar to that formed by hippuric acid. Samples of urine were hydrolyzed with concentrated HCl and partitioned between the aqueous and organic phases. Most of the radioactivity was found in the organic fraction, and TLC analysis showed the presence of a single component more polar than BMIPP and AMIPT. Analysis by HPLC (Fig. 7) showed the presence of ^{125}I- and ^{131}I-labeled components that showed the same retention times as PIPA.

DISCUSSION

The availability of the 3(S)- and 3(R)-BMIPP isomers and authentic samples of AMIPT and PIPA has provided an opportunity to evaluate the relative metabolism of the two isomers. Initial observations (*17,18*) showing increased heart uptake of the 3(R)-BMIPP isomer compared with 3(S)-BMIPP in rats suggested that this difference might result from differences in the relative myocardial metabolism of the two isomers, because the prolonged retention observed with racemic BMIPP has been shown to be associated with incorporation into intracellular TG storage lipids. Studies here have shown, however, that there are no major differences in the distribution of radioactivity in tissue lipids in rat tissues, measured over the period studied.

Both isomers appear to be metabolized in the same manner. The results also show that the relative radioactive contents of both urine and feces are the same after administration of both 3(R)- and 3(S)-BMIPP, and both radioisotopes are excreted at the same rate. By TLC analysis, the radioactive contents of the urine consisted of a single radioactive component with the same mobility as hippuric acid. Because HPLC analysis of the organic fraction ob-

tained by acid hydrolysis of urine contained exclusively PIPA, these results suggest that the short-chain PIPA (*30*) is probably conjugated with an amino acid to facilitate renal clearance, similar to the excretion of benzoic acid as the amide conjugate with glycine.

HPLC analysis of the FFA fraction and the acid fraction released by basic hydrolysis of the myocardial lipids showed a mixture primarily consisting of fatty acids with chain lengths less than those of BMIPP. In earlier studies (*1–6,21–23,25–27*), only the total TG fraction was evaluated, without analysis of the nonsaponifiable fraction released by basic hydrolysis. Figure 8 summarizes the proposed metabolism, based on the results of these studies, of BMIPP in rat myocardium. It appears that the various oxidation products can be shunted into the TG storage pool. We have not observed the presence of the final PIPA in either the free acid pool or the acid fraction released from TG hydrolysis. PIPA must therefore not accumulate in the myocardial cells but must immediately leave the myocytes after formation.

Although the two BMIPP isomers appear to be metabolized in exactly the same way in rat tissues, at least two major questions remain unanswered regarding the metabolism of BMIPP. The first question encompasses the intracellular kinetics of transfer of the initial α-oxidation product (Fig. 8) from the initial site of oxidation (peroxisomes?) to the site of subsequent β-oxidation, which presumably occurs in the mitochondrial compartment (*31*). The peroxisome has been identified as the site for α-oxidative metabolism of long-chain fatty acids (*31,32*). The second question concerns the mechanism of fatty acid uptake by the myocytes. If the differences in relative myocardial uptake of 3(R)- and 3(S)-BMIPP result from transport from the interstitial space through the myocyte membrane, such differences must reflect differences in binding to membrane-bound fatty acid–binding proteins (*33–36*).

CONCLUSION

Although the relative myocardial uptake of 3(R)-BMIPP is 20%–25% greater than that of 3(S)-BMIPP, this study has shown no apparent differences in the lipid pool distribution of the isomers during the 15–180 min time frame studied in rats. The reasons for the enhanced myocyte uptake of 3(R)-BMIPP observed in rats in comparison with 3(S)-BMIPP are unclear, but this observation may result from differences in the relative membrane translocation of the two isomers from the intravascular space into the myocytes. Fortunately, this difference is not observed in humans (*19*). These results are important in expanding our understanding of the metabolism of BMIPP. The reasons for this observed difference in small animals remain to be found.

ACKNOWLEDGMENTS

Research at the Oak Ridge National Laboratory (ORNL) is supported by the Office of Biological and Environmental Research (OBER), U.S. Department of Energy (DOE), under contract DEAC05–96OR2464 with Lockheed Martin Energy Research Corporation. Qun Lin acknowledges support from the Historically Black Colleges and Universities Faculty Research Participation Program sponsored by the OBER, U.S. DOE, and administered by the Oak Ridge Institute for Science and Education (ORISE) for research conducted in the Nuclear Medicine Program at ORNL in 1995. Huimin Luo acknowledges support for research conducted at ORNL from the Alexander Hollaender Distinguished Postdoctoral Fellowship Program administered by ORISE. Florian T. Mokler thanks the Heinrich J. Klein Foerderstiftung in Mainz, Germany, for partial support during his 6-mo stay at ORNL during the 1995–1996 period, for the research completed in partial fulfillment for the thesis research requirements for the medical degree at the University of Mainz. This article contains a major part of his thesis research. The authors also thank Dr. Yoshihiro Yamamichi and colleagues from the Nihon Medi-Physics Central Research Laboratory in Japan for providing authentic samples of PIPA, AMIPT and the C_{12}, C_{10} and C_6 chain-length metabolites.

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