Metabolism of Iodine-123-BMIPP in Perfused Rat Hearts

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Increased clinical use of ^{123}I-labeled 15-(*p*-iodophenyl)-3-(R,S)-methyl-pentadecanoic acid ([^{123}I]BMIPP) revealed discordance between BMIPP uptake and that of perfusion agents, which was inexplicable due to the uncertainty of its myocardial metabolism. This study clarifies the metabolic fate of BMIPP and its relation to substrates in isolated rat hearts. **Methods:** Rat hearts were perfused with 5 mmole/liter HEPES buffer containing various energy substrates and 1% bovine serum albumin. The buffer was recirculated for 4 hr after bolus injection of [^{123}I]BMIPP. Heart time-activity curves were monitored externally. After perfusion, the radioactivity in the heart and recirculated buffer was measured. The metabolites in the buffer were then extracted and analyzed by HPLC and TLC. **Results:** When 0.4 mmole/liter oleate was the energy substrate, more than eight radioactive BMIPP metabolites were detected. The metabolites in the coronary effluent depended on the energy substrate in the buffer. The radioactivity in the heart at the end of the perfusion period was significantly higher when 0.4 mmole/liter oleate (28.0% ± 1.2% ID/g, mean ± s.e.m.) or 10 mmole/liter glucose with 25 U/liter insulin (43.9% ± 2.2% ID/g) were the substrates compared to when 5 mmole/liter acetate (8.5% ± 0.4% ID/g) or 0.4 mmole/liter cold BMIPP (6.2% ± 0.3% ID/g) were the substrates. The distribution of metabolites suggests that oleate stimulated both alpha and beta oxidations, whereas glucose with insulin inhibited both. Acetate also stimulated alpha oxidation but not beta oxidation. Cold BMIPP strongly inhibited both alpha- and beta-oxidations, and little alpha oxidation occurred compared to beta-oxidation. **Conclusion:** These results suggest that [^{123}I]BMIPP is metabolized in the myocardium and the metabolism is closely related to myocardial carbohydrate utilization.

Key Words: fatty acid metabolism; iodine-123-BMIPP; alpha oxidation; beta oxidation; myocardial perfusion agents

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Iodine-123-labeled beta-methyl-*p*-iodophenylpentadecanoic acid ([^{123}I]BMIPP) is a radioiodinated fatty acid developed for use as a myocardial imaging agent (*1*). It was designed to achieve prolonged retention in the myocardium compared with the corresponding straight-chain analogs, such as (*p*-iodophenyl)-pentadecanoic acid (IPPA), for SPECT imaging (*2*). As the use of BMIPP increased in clinics, it was recognized that there are complicated discrepancies between BMIPP metabolism and myocardial perfusion in patients with myocardial infarction and hypertrophic cardiomyopathy (*3–9*); these discrepancies, however, are difficult to interpret, because myocardial metabolism of BMIPP is not well understood. Two possible pathways have been proposed for the catabolism of BMIPP (*1*). The first pathway is direct beta-oxidation of BMIPP which causes myocardial accumulation of beta-hydroxy-BMIPP, because further beta-oxidation is inhibited by the branched-methyl group. The second pathway is initial alpha oxidation producing alpha-hydroxy-BMIPP as an intermediate, which would then be degraded through successive cycles of beta-oxidation to (*p*-iodophenyl)-acetic acid as the end product.

It was shown that the BMIPP kinetics in isolated rat hearts is insensitive to changes in the fatty acid oxidation rate caused by a carnitine palmitoyltransferase I inhibitor, 2[5(4-chlorophenyl)-pentyl]oxirane-2-carboxylate (POCA) (*10*). An experiment using cultured embryonal myocytes showed that BMIPP is not metabolized in the myocardium (*11*). In contrast, some metabolites of [^{123}I]BMIPP were detected after intravenous administration to rats, rabbits and humans. In rats, [^{123}I]BMIPP rapidly disappeared from the blood, and ^{123}I-*p*-iodophenylacetic acid (PIPA) or its conjugate with glycine was dominant in the blood as early as 5 min after injection. In rabbits and humans, the disappearance of [^{123}I]BMIPP was slightly slower than in rats, and only PIPA was detected in the blood (*12*). The radioactive metabolite in the urine was mostly a conjugate of PIPA with glycine in rats, a conjugate with gluconic acid in rabbits, and conjugates with gluconic acid and glutamine in humans (*12*). PIPA was the end product of [^{123}I]BMIPP metabolism, and it was possibly generated via initial alpha oxidation and subsequent beta-oxidation, as predicted in previous reports (*1,13*); however, these metabolites re-

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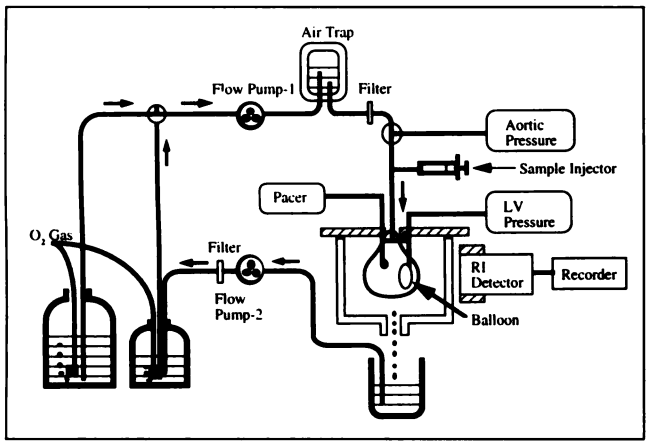

FIGURE 1. Schematic diagram of perfusion system for isolated rat hearts. RI = radioactive intensity.

flected only the overall catabolism of BMIPP in the whole body and do not, necessarily, indicate the metabolic fate of the tracer in the heart. Recently, investigators detected an unidentified polar metabolite in the effluent of perfused rat hearts that had received ^{125}I-BMIPP (*14,15*). It was speculated that the metabolite was ^{125}I-beta-hydroxy-BMIPP, but it was not identical to the authentic sample. Consequently, it is still unknown whether BMIPP can be metabolized in the myocardium.

This study aims to elucidate the myocardial metabolism of [^{123}I]BMIPP. Metabolites in the coronary effluent were collected by recirculated perfusion of the isolated rat heart to rule out the contribution of other organs and to minimize the loss and dilution of radioactivity. Energy substrate dependency of the metabolism was also examined.

MATERIALS AND METHODS

Male Sprague-Dawley rats (240–300 g body weight) were anesthetized with thiopental sodium (38 mg/kg i.p., Tanabe Seiyaku, Osaka, Japan) and heparinized. After rapid excision of the heart, the aorta was cannulated and retrogradely perfused with a N-2-hydroxyethylpiperazine-N-2-ethanesulfonic acid (HEPES) buffer composed of the following, equilibrated with 100% O_2: 123 mmole/liter NaCl, 5 mmole/liter KCl, 1 mmole/liter $MgSO_4$, 1.5 mmole/liter $CaCl_2$, 5 mmole/liter sodium acetate and 6 mmole/liter glucose. The pH of the perfusate was adjusted to 7.4 at 37°C by adding NaOH. The perfusion system is illustrated in Figure 1. The heart contracted spontaneously at 2.5–3.5 Hz. When the heart rate became slower than 2.5 Hz, contraction was maintained by right ventricular pacing. A latex balloon tied to the end of a polyethylene tube was passed into the left ventricle through the mitral valve and connected to a pressure transducer (P23XL, Viggo-Spectramed, Oxnard, CA). The balloon volume was set to achieve an initial end-diastolic pressure of 4–10 mmHg throughout the experiments. The left ventricular pressure and perfusion pressure were recorded with a direct-writing recorder (WindoGraf, Gould, Valley View, OH). The coronary flow rate, controlled by a peristaltic pump, was adjusted so that the perfusion pressure was about 70 mmHg, then kept constant. After 20–30 min of stabilization, the perfusate was switched to a test solution containing 1% bovine serum albumin (BSA) and one of the following energy substrates: 0.4 mmole/liter oleate, 10 mmole/liter glucose with 25 U/liter insulin, 5 mmole/liter acetate, or 0.4 mmole/liter cold BMIPP with 0.4 mmole/liter oleate. BSA was used to make complexes with the fatty acids contained in each buffer and also to stabilize [^{123}I]BMIPP during the perfusion. Oleate was chosen as a typical fatty acid because it was not only the most dominant component in the plasma, but was also able to make stable complexes with BSA (*16*). Acetate was used to study the metabolism of BMIPP when fatty acid utilization was completed. Glucose with insulin was used to study the metabolism of BMIPP under the increased glucose-loading conditions. Carrier dependency on the metabolism was examined by addition of cold BMIPP.

About 10 min after switching from the perfusate to the test solution, the heart reached a new steady-state. Then flow pump 2 (Fig. 1) recirculated the effluent and [^{123}I]BMIPP (37 MBq) complexed with BSA was injected as a bolus into the perfusion line. The perfusion was continued for 4 hr after the tracer injection. The time-activity curve was determined using a 1 × 1 in. NaI(Tl) scintillation probe (Steffi, Raytest Inc., Straubenhardt, Germany) located 4 cm from the heart and fitted with a 7-mm thick lead shield. The count rate was recorded using a D-2500 Chromato integrator (Hitachi Co., Ltd., Tokyo, Japan). At the end of the experiment, the radioactive contents of the heart and perfusate collected from the circulation system were measured using a 2 × 2 in. NaI(Tl) scintillator interfaced to a single channel analyzer (Ohyo Koken Kogyo, Tokyo, Japan).

Analysis of Effluent Metabolites

The total effluent (ca. 200 ml) recovered from the perfusion system was acidified with hydrochloride and extracted twice with four volumes of a mixture of chloroform and methanol (2:1) using the same method as Folch (*17*). This procedure extracted more than 99% of the radioactivity. The organic phase was evaporated to dryness and redissolved in a few ml of methanol for analysis using HPLC or TLC. To analyze the time course of the metabolites, a 4-ml aliquot of the effluent was taken from the recirculated perfusate at 10 min, and 1, 2, 3 and 4 hr after the injection of [^{123}I]BMIPP and extracted as described previously.

HPLC analysis of the extracted sample was performed on a preparative column (YMC-pack, ODS-A, 15 cm × 2 cm ID; YMC Co., Ltd., Kyoto, Japan). The radiochromatogram was developed with methanol:water:acetic acid (96:4:1) at a flow rate of 6.0 ml/min. The detected radioactive peaks were quantified by integrating the output of the single channel analyzer. Metabolites were identified by comparison of their retention times with those of authentic standards injected simultaneously.

An aliquot of the extract was chromatographed on TLC plates (Silica gel-60; E. Merck, Darmstadt, Germany) using the following two solvent systems: *n*-hexane:diethylether:acetic acid (60:40:1) and chloroform:methanol:acetic acid (90:5:5). The radioactivity was measured with a Bio-imaging analyzer (BAS2000, Fuji Photo Film, Tokyo, Japan). The radio-peaks fractionated by HPLC were also analyzed by TLC and identified by comparison with the migration of authentic standards after staining with iodine.

Reagents

Cold BMIPP and PIPA were synthesized as described in previous reports (*18,19*). *p*-Iodophenyldodecanoic acid ($PIPC_{12}$) and *p*-iodophenylhexanoic acid ($PIPC_6$) were purchased from Med-Life Systems Inc. (Upper Darby, PA). Sodium oleate and *p*-Iodophenylbenzoic acid (PIBA) were purchased from Nacalai Tesque, Inc. (Kyoto, Japan). BSA (fraction V, fatty acid-free) was purchased from Miles Inc. (Kankakee, IL). All other reagents were

obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan) or Tokyo Kasei Kogyo Co., Ltd. (Tokyo, Japan).

The [^{123}I]BMIPP used in this study was a commercially available product (Nihon Medi-Physics Co., Ltd., Nishinomiya, Hyogo, Japan). Its radiochemical purity was more than 98%, and the concentration of its radioactivity was 74 MBq/ml at calibration time (0.4 mg/ml of carrier BMIPP was contained). Prior to use, it was dissolved in a HEPES buffer containing 1% BSA, and the complex formation of [^{123}I]BMIPP with BSA was confirmed by electrophoretic analysis (1 mA/cm, 30 min, 0.06 M barbital buffer, pH 8.6). The radio-peak of [^{123}I]BMIPP coincided in migration distance to the spot of BSA stained by ponceau 3R on the cellulose acetate strip.

The major compounds in the metabolism of BMIPP were selected on the basis of the hypothetical metabolic pathways (*1,13*) and were synthesized as authentic standards. To synthesize 15-(*p*-iodophenyl)-2-(R,S)-chloro-3-(R,S)-methylpentadecanoic acid (α-Cl-BMIPP), cold BMIPP was alpha chlorinated with N-chlorosuccinimide (*20*). Unlabeled BMIPP (0.458 g, 1 mmole/liter) was stirred at 70°C for 2 hr in 0.5 ml of thionyl chloride, and then N-chlorosuccinimide (0.343 g, 2.6 mmole/liter) in 20 ml of carbon tetrachloride, 0.2 ml of thionyl chloride and concentrated HCl (0.01 ml) were added. The resulting solution was refluxed for 2 hr while stirring. After cooling to room temperature, volatile materials were evaporated and the residue was extracted with carbon tetrachloride. The combined organic extracts were dried over anhydrous Na_2SO_4, and the solvent was removed in vacuo to give 0.419 g of alpha-Cl-BMIPP (85%) as a yellow oil [^{1}H-NMR ($CDCl_3$); ppm: 1.0–1.1(dd,3H), 1.25(s,20H), 1.60(m,2H), 2.55(t,2H), 4.45, 4.62(m,1H), 6.90(d,2H), 7.58(d,2H). TLC(silica gel $60F_{254}$, hexane:ethylacetate = 5:1); Rf = 0.2].

To synthesize 15-(*p*-iodophenyl)-2-(R,S)-hydroxy-3-(R,S)-methylpentadecanoic acid (α-OH-BMIPP), 2 ml of 2N NaOH was added to a solution of α-Cl-BMIPP (0.198 g, 0.40 mmole/liter) in 4 ml of ethanol, and the mixture was refluxed for 3 hr. After the ethanol was evaporated, the resulting mixture was acidified to pH 3 with dilute HCl and extracted with ether. The organic layer was dried over anhydrous Na_2SO_4 and the solvent was removed to yield an oil. The crude product was purified on a preparative silica gel plate developed with chloroform:methanol = 5:1(Rf = 0.5) to yield 60 mg of α-OH-BMIPP (31%) as an oil [^{1}H-NMR (CD_3OD); ppm: 0.96(d,3H), 1.30(s,20H), 1.60(m,2H), 2.15(m,1H), 2.55(t,2H), 4.0–4.2(m,1H), 6.95(d,2H), 7.58(d,2H)].

To synthesize 14-(*p*-iodophenyl)-2-(R,S)-methyltetradecyl aldehyde (AMIPT-CHO), α-hydroxycarboxylic acid was decarboxylated to the corresponding noraldehydes (*21*). A solution of α-OH-BMIPP (96 mg, 0.2 mmole/liter) and tetrabutylammonium periodate (104 mg, 0.25 mmole/liter) in 5 ml of chloroform was refluxed for 13 hr. The mixture was cooled to room temperature, poured into 50 ml of 10% $Na_2S_2O_3$ solution and extracted with chloroform. The combined chloroform extracts were dried over anhydrous Na_2SO_4, and the solvent was removed to yield an oil. The crude product was purified by silica gel column chromatography with *n*-hexane:ethylacetate = 10:1 to 5:1 as eluents to give 44 mg of AMIPT-CHO [^{1}H-NMR($CDCl_3$) ppm: 1.05(d,3H), 1.30(s,20H), 1.60(m,2H), 2.35(m,1H), 2.55(t,2H), 6.90(d,2H), 7.60(d,2H), 9.60(d,1H)].

To synthesize 14-(*p*-iodophenyl)-2-(R,S)-methyltetradecanoic acid (AMIPT), selective oxidation of the aldehyde to the carboxylic acid was performed with sodium chlorite-hydrogen peroxide (*22*). Sixteen milligrams of $NaH_2PO_4 \cdot H_2O$ in 0.1 ml of H_2O, 0.02 ml of 30% H_2O_2 and 16 mg of $NaClO_2$ in 0.5 ml of H_2O were added to AMIPT-CHO (44 mg, 0.1 mmole/liter) in 1 ml of acetonitrile while stirring at 10°C. The resulting mixture was then stirred at 10°C for 2 hr. After quenching with 1 mg of Na_2SO_3, the mixture was acidified with 3 ml of 1*N* HCl and concentrated in vacuo. The resulting residue was extracted with chloroform. The organic layer was dried over anhydrous $MgSO_4$, and the solvent was removed to yield an oil. The crude product was purified on a preparative silica gel plate developed with chloroform:methanol = 5:1 (Rf = 0.7) as an eluent, thereby yielding 16 mg of AMIPT (35%) as a white solid (mp 55–57°C) [^{1}H-NMR($CDCl_3$) ppm: 1.19(d,3H), 1.25(s,20H), 1.60(m,2H), 2.45(m,1H), 2.54(t,2H), 6.91(d,2H), 7.58(d,2H). FABMS; 443(M-H)$^-$, HRFABMS calculated for $C_{21}H_{32}IO_2$ 443.1445, found 443.1489].

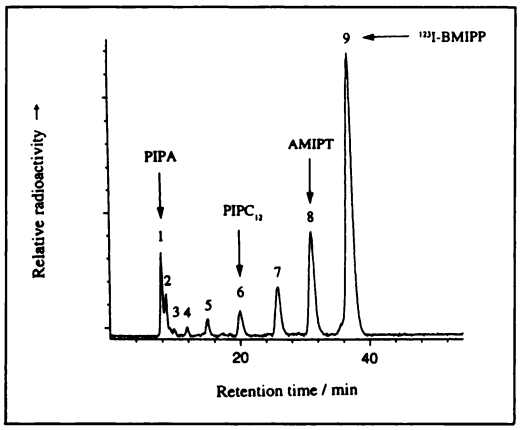

FIGURE 2. A typical radio-profile of high performance liquid chromatography of the effluent from the heart perfused with 0.4 mmole/liter oleate. The perfusate was recirculated for 4 hr after the administration of [^{123}I]BMIPP.

Statistical Analysis

Data are presented as the mean ± s.e.m. Statistical analysis was performed using the paired t-test or one-way analysis of variance (ANOVA). The probability of the null hypothesis less than 5% was considered significant.

RESULTS

Identification of BMIPP Metabolites in Myocardium

Figure 2 shows a typical radio-HPLC profile of the effluent from the heart perfused with 0.4 mmole/liter oleate as an energy substrate for 4 hr after the injection of [^{123}I]BMIPP. The major radioactive peak eluting at 36.5 min (peak 9) was the unchanged form of [^{123}I]BMIPP, which was not taken up into or back-diffused from the heart, so that the other eight peaks (peaks 1 to 8) eluting before the parent peak were myocardial metabolites. In preliminary experiments, the HPLC did not detect any radiochemical impurities in the [^{123}I]BMIPP preparation. The height of the peaks from 3 to 8 increased with the peak number, whereas peaks 1 to 3 decreased in this order. Three radiopeaks eluting at 8.6 min (peak 1), 20.4 min (peak 6) and 31.0 min (peak 8) were identified by comparison of their retention times with the authentic standards on HPLC (Table 1), and they were PIPA, ^{123}I-PIPC$_{12}$ and ^{123}I-AMIPT, respectively. The identities of these metabolites were also con-

TABLE 1
HPLC and TLC Properties of Authentic Standards

Authentic standard	HPLC (Rt/min)	TLC1 (Rf)*	TLC2 (Rf)†
p-Iodophenylacetic acid (PIPA)	8.6	0.25	0.70
p-Iodobenzoic acid (PIBA)	9.1	0.28	0.70
p-Iodophenylhexanoic acid ($PIPC_6$)	10.5	0.30	0.73
p-Iodophenyldodecanoic acid ($PIPC_{12}$)	20.4	0.44	0.77
α-Methyl-*p*-iodophenyltetradecanoic acid (AMIPT)	31.0	0.54	0.79
p-Iodophenylpentadecanoic acid (IPPA)	32.4	0.46	0.78
β-Methyl-*p*-iodophenylpentadecanoic acid (BMIPP)	36.5	0.53	0.79
α-Hydroxy-β-methyl-*p*-iodophenylpentadecanoic acid (α-OH-BMIPP)	38.0 + 39.1 (1:4)	0.28	0.69

*TLC1: *n*-hexane:diethylether:acetic acid (60:40:1), silica gel-60.
†TLC2: chloroform:methanol:acetic acid (90:5:5), silica gel-60. HPLC = high-performance liquid chromatography; TLC = thin-layer chromatography.

firmed using TLC analyses developed with two solvent systems (Fig. 3).

AMIPT, which was the major metabolite, is the product of alpha-hydroxylation followed by oxidative decarboxylation of [^{123}I]BMIPP. $PIPC_{12}$ and PIPA are the products of the first cycle and the final cycle of beta-oxidation, respectively. Thus, these results indicate that [^{123}I]BMIPP was metabolized via initial alpha-oxidation and subsequent cycles of betaoxidation. Peak 3, eluting at 10.0 min in Figure 2, showed similar properties to ^{123}I-*p*-iodophenylhexanoic acid ($PIPC_6$), but its identity remained uncertain. Although alpha-OH-BMIPP was presumed to exist as one of the metabolites, no radioactive peak was found at the corresponding retention time position on the HPLC. The rest of the radioactive species (peaks 2, 4, 5 and 7) have not yet been identified.

The formation of ^{123}I-*p*-iodobenzoic acid (PIBA) was suspected because the retention time (9.3 min) of peak 2 (Fig. 2) was very close to that of the authentic standard PIBA (Table 1); however, this was ruled out because their Rf values on TLC1 and retention times on HPLC were different when the fractionated metabolite and the authentic standard were simultaneously examined.

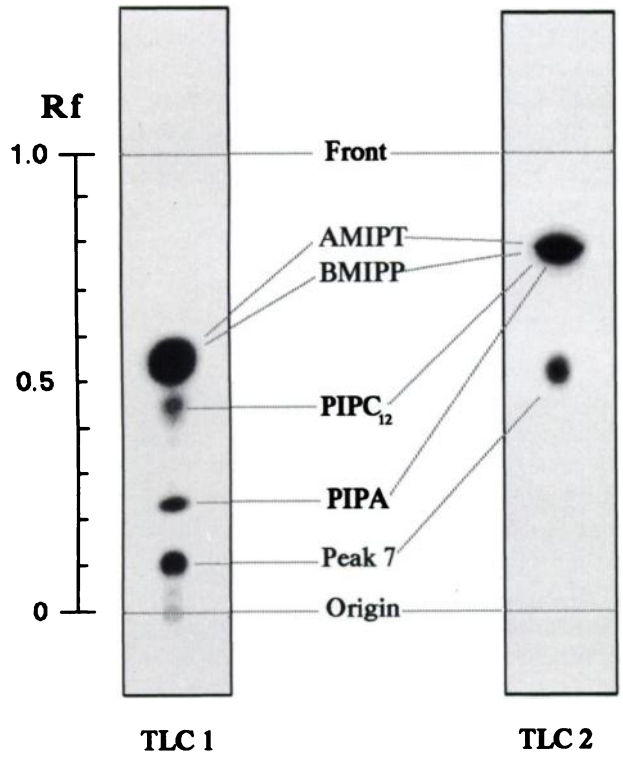

FIGURE 3. Radio-profile of TLC of the effluent from the heart perfused with 0.4 mmole/liter oleate, which was obtained from the same sample used for high performance liquid chromatography analysis as in Figure 2. TLC1 was developed with *n*-hexane:diethylether:acetic acid (60:40:1), while TLC2 was developed with chloroform:methanol:acetic acid (90:5:5).

Time Course of Metabolism

Figure 4 shows the change in the contents of [^{123}I]BMIPP metabolites in the effluent during 4 hr of perfusion. Iodine-123-BMIPP decreased with time to 58.6 ± 2.8% of the total radioactivity in the effluent (n = 3, Fig. 4A), whereas the metabolites increased cumulatively (Fig.

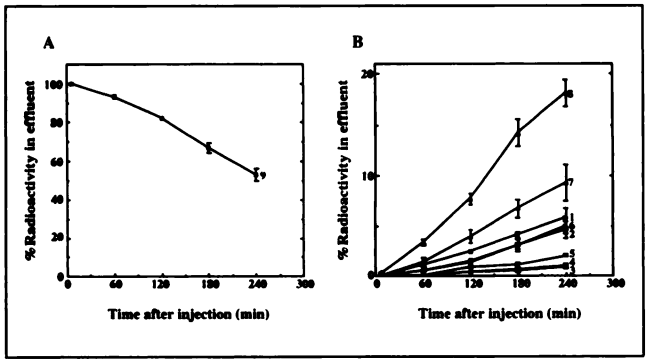

FIGURE 4. Time course of metabolites in the effluent containing 0.4 mmole/liter oleate as the energy substrate. Hearts were perfused for 4 hr after the injection of [^{123}I]BMIPP. Aliquots of the perfusate were taken at 10 min, and 1, 2, 3 and 4 hr after the injection. Sampling loss of the effluent volume was not corrected (n = 3). (A) Unchanged BMIPP. (B) Metabolites. The number of lines corresponds to the number of peaks in Figure 2.

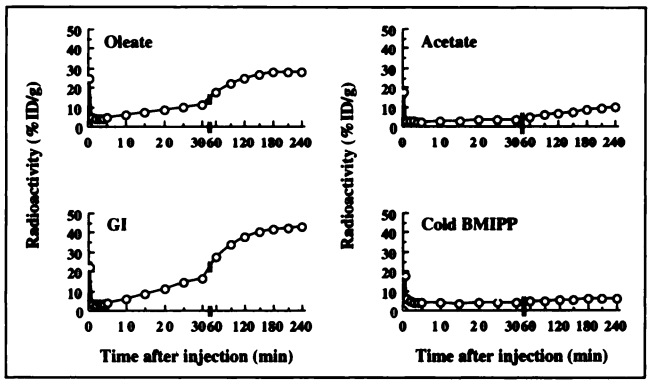

FIGURE 5. Time-activity curve of the heart using different substrates after the injection of [^{123}I]BMIPP. Uptake value was corrected for the half-life of ^{123}I. ID = injected dose.

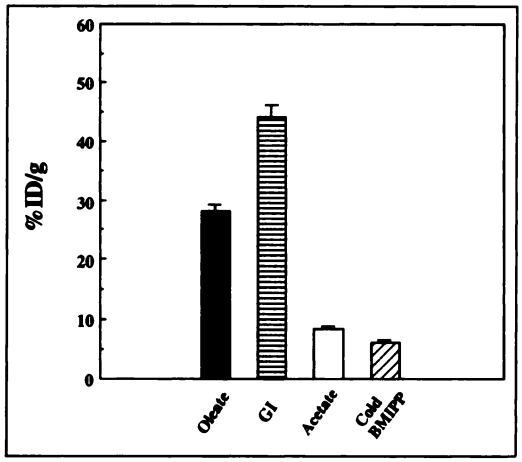

FIGURE 6. Heart uptake of radioactivity at the end of the perfusion period (n = 6 for oleate; n = 5 for the other groups). ID = injected dose; GI = glucose and insulin solution.

4B). We did not take into consideration the possibility that the metabolites, once released into the effluent, might be susceptible to repeated uptake by the myocardium.

Effects of Substrates on BMIPP Metabolism

Figure 5 presents typical time-activity curves of the hearts perfused with the various energy substrates. Within 1 min after bolus injection of the tracer, peaks appeared, and 2%–6% of the radioactivity was taken up in the heart (initial heart uptake). Then, the radioactivity of the hearts either increased gradually or reached a certain equilibrium state during the recirculation with the tracer, depending on the energy substrate. There was no significant difference in the initial heart uptake between the group administered glucose with insulin (3.1% ± 0.2% ID/g wet wt, n = 5) and the oleate group (3.9% ± 0.4% ID/g, n = 6), but the heart uptake in the glucose-insulin group became greater than that in the oleate group after 20 min of recirculation. In the acetate group, the hearts showed smaller initial uptake (2.4% ± 0.3% ID/g, n = 5) than the oleate group ($p < 0.05$) and little increase in the uptake during the recirculation. The initial uptake in the cold BMIPP group (5.7% ± 0.6% ID/g, n = 5) was greater than that in the oleate group ($p < 0.05$), but the heart uptake remained at a low level throughout the experiment.

Figures 6 and 7 show the heart uptakes (% ID/g wet wt, n = 5 or 6) at the end of the perfusion period and the distribution of the metabolites and the parent (% ID, n = 5 or 6) in the effluents recirculated for 4 hr with four different energy substrates, respectively. In the glucose-insulin group, the heart uptake (43.9% ± 2.2% ID/g) was significantly greater than that in the oleate group (28.0% ± 1.2% ID/g, $p < 0.001$, Fig. 6). It had been presumed that there would be less uptake in the heart in the glucose-insulin group than in the oleate group, because fatty acid utilization would fall in the glucose-insulin group; the actual results, however, are contrary to that presumption, except for the initial uptake phase (Fig. 5). In spite of greater heart uptake in the glucose-insulin group, the sum of the metabolites (peaks 1 to 8) was smaller than that in the oleate group ($p < 0.001$), probably because perfusion with glucose and insulin inhibited fatty acid oxidation (Fig. 7). It is not clear why peaks 3 to 5, which may be attributable to middle chain-length intermediates, were not detected, although, both PIPA (the end product) and $PIPC_{12}$ (product of the first cycle of beta oxidation) appeared (Fig. 7).

In the acetate group, the heart uptake (8.5% ± 0.4% ID/g) was significantly smaller than in the oleate group ($p < 0.001$, Fig. 6), and the sum of the percentage of the metabolites (peaks 1 to 7) except AMIPT was also lower ($p < 0.01$, Fig. 7). These results indicate that acetate is utilized as the principal energy source prior to fatty acids, since acetate is a direct energy source for oxidative phosphorylation.

The cold BMIPP group showed the smallest heart uptake (6.2% ± 0.3% ID/g) among the four groups ($p < 0.001$), and no metabolites were detected except for a small amount of PIPA (0.2% ± 0.1% ID/g). When cold BMIPP

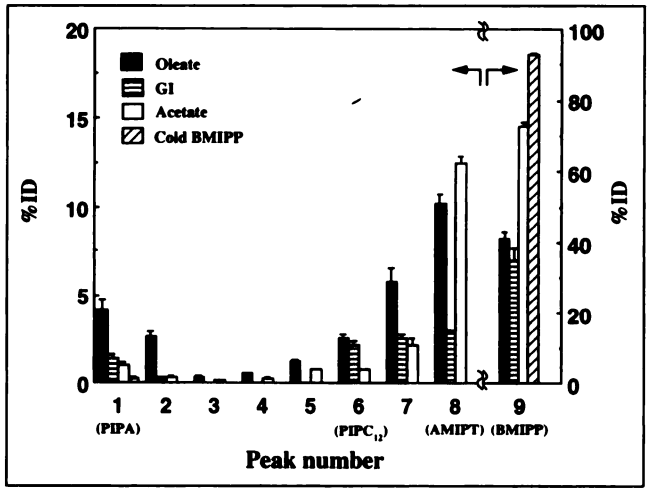

FIGURE 7. Distribution of the metabolites and the parent (BMIPP) in the effluents recirculated for 4 hr with different energy substrates (n = 6 for oleate; n = 5 for the other groups). GI = glucose and insulin; ID = injected dose.

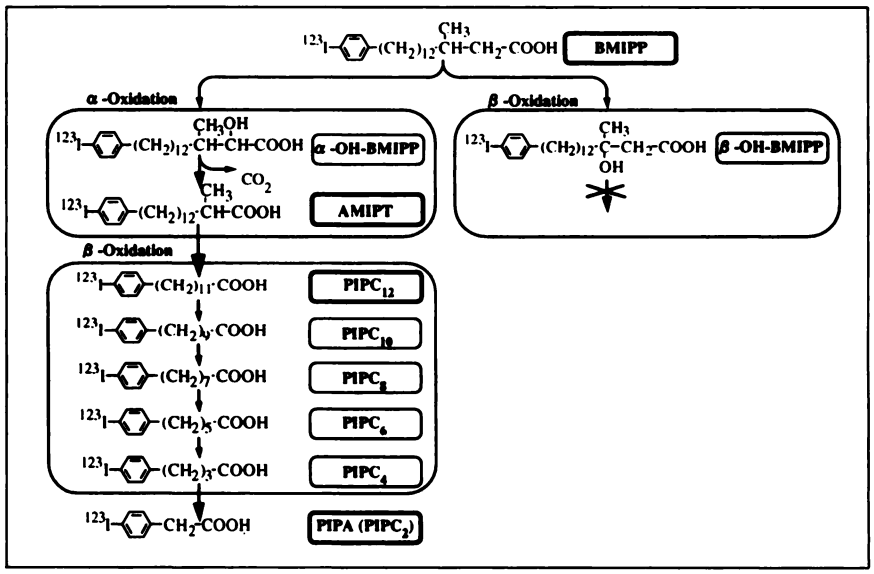

FIGURE 8. Metabolic pathway of [^{123}I] BMIPP in rat heart suggested by the findings of this study. Metabolites surrounded by a thick line were detected and identified by both HPLC and TLC analyses. Metabolites surrounded by a thin line have not yet been detected or identified but are expected to be formed during the catabolic process.

was used as the sole energy source in the perfusate, the developed pressure of the heart rapidly fell to one third of the control within 30 min (data not shown). In contrast, in the cold BMIPP group, which used 0.4 mmole/liter cold BMIPP and 0.4 mmole/liter oleate as the energy sources, the hearts showed stable generation of pressure. This result suggests that the rat heart can hardly use such a macro amount of cold BMIPP as an energy source, and that cold BMIPP blocks neither the uptake of the usual fatty acids nor their beta-oxidation.

DISCUSSION

Metabolism of BMIPP in the Myocardium

BMIPP is thought to be incorporated into the myocardium largely depending on regional blood flow, which is similar to incorporation of other fatty acids, but BMIPP is protected from beta-oxidation by the methyl-branched group. As the use of BMIPP has increased in clinics, it has been recognized that there are complicated discrepancies between BMIPP metabolism and myocardial perfusion in patients with myocardial infarction and hypertrophic cardiomyopathy (*3–9*); however, BMIPP metabolism was difficult to interpret because BMIPP metabolism is not well understood.

Three BMIPP metabolites, AMIPT, $PIPC_{12}$ and PIPA, were identified in this study. These are key compounds supporting one of the previous predictions for the metabolic pathway of [^{123}I]BMIPP (Fig. 8) (*1,13*). AMIPT in the effluent was always the most abundant metabolite (Fig. 7), which strongly suggests that [^{123}I]BMIPP is first digested by alpha-oxidation similar to phytanic acid, an analog of natural fatty acids with beta-methyl substituents (*23,24*). Although [^{123}I]BMIPP would be initially alpha-hydroxylated and then decarboxylated to AMIPT, the alpha-hydroxylated intermediate (alpha-OH-BMIPP) was not detected in our study, probably due to enzymatic channeling in the hydroxylation process (*23*). AMIPT would subsequently be processed by classic beta-oxidation since it would no longer be impeded by the branched-methyl group, now converted from the beta position to the alpha position. PIPA could be the end product of AMIPT through successive beta-oxidation cycles, which suggests the occurrence of complete chain-shortening of [^{123}I]BMIPP in the rat heart. The appearance of $PIPC_{12}$, which is generated from AMIPT by one cycle of the beta-oxidation process, indicates that such intermediates permeate from myocardial cells, as AMIPT does. The release of a series of chain-shortened fatty acids was also observed in isolated rat liver when incubated with omega phenyllauric acid (*25*). These results can be explained by steric hindrance of the terminal phenyl or iodophenyl groups, which may slow down the enzymatic reaction compared with the corresponding aliphatic fatty acids (*26*). Although it is uncertain whether such intermediate chain-length fatty acids are also eliminated from the myocardium in vivo, none were detected in the blood or urine of rats in an in vivo study, probably due to rapid reuptake by the liver and soft tissues (*12*).

It has also been proposed (*27*) that the branched-methyl group may be cleaved at the beta position by a biotin-catabolized reaction and transformed to ^{123}I-*p*-iodophenyl-pentadecanoic acid (^{123}I-IPPA); however, we did not detect a radioactive-peak corresponding to the metabolite resulting from this reaction (Table 1) that would suggest that this pathway is unlikely. It is also conceivable that an omega oxidation or some other random oxidation might lead to the same end product as beta-oxidation. Indeed, β-methyl-l-^{14}C-heptadecanoic acid (BMHDA) is mainly processed by omega-oxidation (66%) rather than alpha-oxidation (<1%) in rat hearts, whereas alpha-oxidation (53%) is a major pathway in the liver (*28*).

When a series of omega-phenyl fatty acids with a straight side chain of 3 to 10 carbon atoms were administered to dogs, the sole end product was either benzoic acid or phenylacetic acid (*29*). Iodine-123-PIBA was not detected

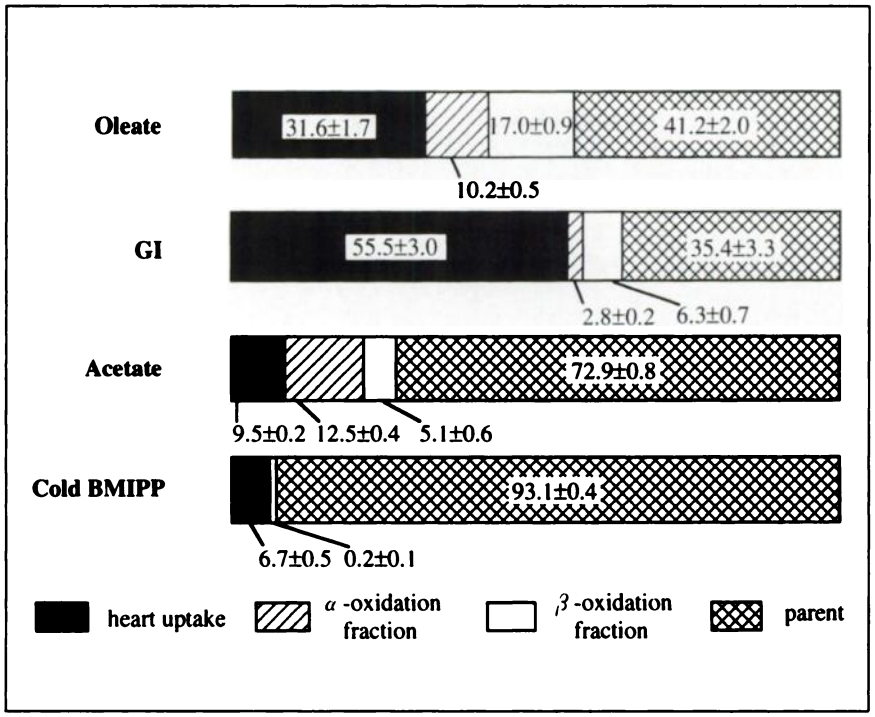

FIGURE 9. Radioactivity distribution at the end of the experiments using various energy substrates (n = 6 for oleate; n = 5 for the other groups). Heart uptake is represented by the radioactivity remaining at the end. The alpha and beta oxidation fractions are expressed by the amount of AMIPT and sum of the amounts of other metabolites (peaks 1 to 7), respectively. The parent is unchanged [^{123}I]BMIPP. GI = glucose and insulin.

in our present study or in an earlier in vivo study (*12*). These results strongly indicate that neither omega-oxidation nor random-oxidation is involved in the degradation of ^{123}I-BMIPP in the rat heart. It was previously indicated (*14,15*) that beta-OH-BMIPP, a possible metabolic trapping product produced by direct beta-oxidation of [^{123}I]BMIPP, was not detected in the myocardial effluent of rats. These results suggest that the involvement of direct beta-oxidation is unlikely. Thus, it appears that initial alpha-oxidation is the major pathway of [^{123}I]BMIPP metabolism in the myocardium. Although the other radioactive peaks (i.e., peaks 2, 3, 4, 5 and 7) have not been identified, it is reasonable to consider that they are intermediates of the beta-oxidation spiral. Further experimental confirmation using authentic standards is necessary.

Dependency of BMIPP Metabolism on Energy Substrate

Our results indicate diversity of the metabolic rate of BMIPP as a function of the energy substrate in the perfusate (Fig. 7). Figure 9 shows the influence of the various energy substrates on the BMIPP metabolism using the amount of AMIPT and the sum of the amounts of the other metabolites (peaks 1 to 7) as the indices for the contributions of the alpha-oxidation fraction and the beta-oxidation fraction, respectively. The total myocardial uptake of ^{123}I-BMIPP, defined by the sum of heart uptake, alpha-oxidation fraction and beta-oxidation fraction, was high in the oleate and glucose-insulin groups and low in the acetate and cold BMIPP groups. Both the oleate and glucose-insulin groups showed very high total uptake, and the initial heart uptake did not differ between the two groups (Fig. 5); however, BMIPP metabolism was significantly different between these two groups. Oleate stimulated both alpha-oxidation (10.2 ± 0.5% ID) and beta-oxidation (17.0 ± 0.9% ID) of [^{123}I]BMIPP. In contrast, each fraction of the alpha-oxidation (2.8 ± 0.2% ID) and the beta-oxidation (6.3 ± 0.7% ID) in the glucose-insulin group was significantly smaller than in the oleate group ($p < 0.001$). Moreover, the heart uptake in the glucose-insulin group was significantly greater than that in the oleate group ($p < 0.001$). These results suggest that the glucose-insulin solution reduced fatty acid ([^{123}I]BMIPP) oxidation due to an increase in glucose consumption, and that the decrease in both oxidation fractions results in an increase in heart retention. This is consistent with earlier observations with ^{11}C-labeled palmitate and ^{123}I-labeled iodohexadecanoic acid (*30,31*). The radioactivity retained in the heart may enter a slow turnover pool.

The acetate group showed moderate total myocardial uptake of [^{123}I]BMIPP (Fig. 9); in spite of low total uptake, the alpha-oxidation fraction was significantly larger (12.5% ± 0.4% ID) compared with that of the oleate group ($p < 0.05$). In contrast, the beta-oxidation fraction (5.1% ± 0.6% ID) was relatively inhibited. These results suggest that acetate inhibits the beta-oxidation of fatty acids due to competition in the acyl-CoA formation (*32*), but that alpha-oxidation is free of such competition. It is also suggested that acetate stimulates alpha-oxidation per se, because the increase of alpha-oxidation fraction was much greater than expected when the beta-oxidation blockade is taken into consideration. In the cold BMIPP group accompanied with 0.4 mmole/liter oleate, the initial heart uptake (5.7% ± 0.6% ID/g) was greater than in the oleate group (3.9% ± 0.4% ID/g, $p < 0.05$, Fig. 5), but the metabolism of [^{123}I]-

BMIPP was completely inhibited, probably due to an extremely small capacity for alpha-oxidation (*28*). The beta-oxidation was also inhibited because it follows the alpha-oxidation. This result suggests that BMIPP is not subjected to another oxidation route such as omega-oxidation or direct beta-oxidation as an alternative to the initial alpha-oxidation. Therefore, the alpha-oxidation process can be considered a rate-limiting step of BMIPP metabolism. Because the heart uptake hardly increased during the perfusion with cold BMIPP (Fig. 5), back-diffusion of [^{123}I]-BMIPP from the myocardium may occur, similar to the case of palmitate (*33*).

It was presumed that peroxisomal beta-oxidation would participate in [^{123}I]BMIPP metabolism in addition to mitochondrial beta-oxidation, as was proven for the metabolism of the ordinary fatty acids (*34,35*). Although peroxisomal beta-oxidation is considered to be preferable for omega phenylated fatty acids rather than the ordinary fatty acids, the mitochondrial contribution is predominant for ^{123}I-BMIPP metabolism because of the production of PIPA as an end product, which would not be produced by peroxisomal oxidation (*25*).

CONCLUSION

Our results indicate that [^{123}I]BMIPP is metabolized via initial alpha-oxidation and subsequent cycles of beta-oxidation, yielding PIPA as the end product. This finding is consistent with considerable myocardial clearance of BMIPP from 20 min to 3 hr after injections revealed in clinical studies (*9*). The discrepacies between BMIPP uptake and regional myocardial perfusion in patients (*3–9*) may be explained by alterations of usage of energy substrates in heart failure.

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