Tracer Kinetics of 15-(Ortho-$^{123/131}$I-Phenyl)-Pentadecanoic Acid (oPPA) and 15-(Para-$^{123/131}$I-Phenyl)-Pentadecanoic Acid (pPPA) in Animals and Man

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The human myocardium retains oPPA as opposed to pPPA. Therefore turnover of oPPA was compared with that of pPPA in rat hearts and in man, the latter by using substrates double-labeled with $^{123/131}$I and ^{14}C. Moreover, substrate binding to coenzyme-A was tested in vitro. In rats, oPPA remained mainly in the pool of free fatty acids, as opposed to pPPA, which was metabolized by mitochondrial β-oxidation. Binding to coenzyme-A at maximum was 62% for oPPA, 81% for pPPA and 90% for palmitic acid.

In man, after i.v. and intracoronary injection of double-labeled oPPA, the two radionuclides reappeared together in venous blood and in coronary sinus respectively, in an unchanged ratio but at a significantly lower rate than with pPPA. It can be concluded that oPPA is bound to coenzyme-A and is retained in the cytosolic lipid pool, while pPPA is metabolized by mitochondrial β-oxidation. A dual-tracer application of oPPA and pPPA has the potential of being a specific probe for the function of the carnitine shuttle.

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The first scintigraphic imaging with a radioiodinated fatty acid, iodine-131 (^{131}I) oleate, was described in 1965 (*1*). In 1973, labeling of heptadecanoic acid with ^{123}I in the omega position was initiated in our laboratory. Iodine-123-heptadecanoic acid (^{123}I-IHA) showed the most physiologic behavior (*2,3*) compared to other fatty acid analogs and has now been used in the diagnosis of coronary artery disease (*4*) and in alcoholic and dilatative cardiomyopathies (*5,6*). Still, for obtaining uptake and elimination curves reflecting fatty acid turnover (*7*) a correction for the water-soluble ^{123}I in blood and tissue (*4,8*) is needed.

To avoid a correction for labeled catabolites, 15-(para-^{123}I-phenyl)-pentadecanoic acid (pPPA) was developed (*9,10*). pPPA behaves metabolically quite similarly to PA (*11,12*) although at a slower speed. pPPA is mainly catabolized to ^{123}I-benzoic acid (*13*), which is rapidly removed from the blood as iodo-hippuric acid after intrahepatic conjugation with glycine (*12*).

When PPA is radioiodinated by electrophilic substitution, two-thirds of the product is in the form of the para-isomer and one-third in the form of the ortho-isomer. Initial animal experiments with the ortho-isomer 15-(ortho-I-phenyl)-pentadecanoic acid (oPPA) revealed a different metabolic behavior compared to pPPA in rat myocardium: oPPA cardiac uptake was slightly lower and its elimination rapid (*11*).

In humans, the kinetics of oPPA was surprisingly different: oPPA is readily taken up and retained with strikingly prolonged half-times ($t_{½}$ = 200 min) (*14*). Serum samples taken from examined patients showed a significantly reduced level of iodinated water-soluble catabolites (*15*) in comparison to pPPA (*12*) during the first 20 min after injection.

The long elimination half-time of oPPA suggested a myocardial trapping of the label. The low level of iodinated water-soluble catabolites, compared with pPPA, indicated an impairment in the β-oxidation of oPPA. To find the trapping site of oPPA, the metabolic pathway of physiologic fatty acids had to be considered (*16–18*). We investigated the metabolic pathway of oPPA by two animal studies, one in vitro enzyme test, and two human studies.

MATERIALS AND METHODS

Radiopharmaceuticals

Phenyl-(2-^{14}C)-pentadecanoic acid (407 MBq) with a specific activity of 2 GBq/mmol was provided by Amersham

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Buchler, Buckinghamshire, England. Our laboratory performed the iodination of the phenyl ring with stable iodine and the separation of para- and ortho-isomers by means of HPLC.

A second paired aliquot of oPPA and pPPA was labeled with ^{123}I for i.v. injection in humans and ^{131}I for the other studies. The labeling was performed by electrophilic substitution as described in detail elsewhere (*9*). The specific activity was ~830 GBq/mmol.

For the dual-tracer studies, the above-mentioned 15-(ortho- or para-^{127}I-phenyl)-(2-^{14}C)-pentadecanoic acid was mixed with the corresponding $^{123/131}I$-labeled isomer resulting in a specific activity of 2.0 GBq/mmol for both ^{14}C and $^{123/131}I$.

Animal Studies

In Vitro Rat Heart Perfusion. In total, eight hearts from male fasted Wistar rats were studied. Thirty minutes after induction of pentobarbital anesthesia (120 mg/kg, i.p.) and anticoagulation pretreatment with heparin (250 U, i.v.), the hearts were prepared for a retrograde perfusion, circumventing longer periods of ischemia by an operative procedure described in detail elsewhere (*19*).

The hearts were stimulated at a constant rate of 280 beats/min and perfused according to the standard Langendorff technique without recirculation of the perfusion medium (*20*). After a 20-min equilibration period, tracer infusion was initiated. The tracer was complexed to albumin in solution and palmitic acid added in a physiologic concentration. About 37 kBq/min of oPPA or pPPA was infused for 30 min. Arterial blood pressure was registered and indicated uncompromised myocardial performance in the experiments. Influx samples and effluates were collected at 1-min time intervals. Finally, the hearts were frozen between precooled aluminum blocks.

Aliquots of the influx and of the collected effluate were counted and total infused and effluate activities were calculated.

The hearts were extracted with chloroform-methanol (2:1) (21) and the homogenates were filtered into pointed glass tubes. The filters were washed with 5 ml of the same solvent and an equal volume of double-distilled water was added. The extracts were agitated for 1.5 min and centrifuged another 20 min at 3000 rpm, providing an upper aqueous and a lower organic phase. The filters containing the solid residue of the hearts and aliquots of the obtained extracts of the hearts were counted and total heart activity was calculated.

The extracts of the hearts were evaporated to dryness and redissolved in a small volume of solvent. Samples of the effluate of the different time intervals were extracted in the same way.

Samples of these concentrates of the effluates and the heart extracts and unlabeled reference substances (iodobenzoic acid, iodophenylpropionic acid, dipalmitin, tripalmitin, and cholesteryl oleate) were treated by thin-layer chromatography (TLC) using the following solvent systems: 1) ethyl acetate; 2) diethyl ether/benzene/ethanol/acetic acid (40:50:2:0,2); and 3) diethyl ether/hexane (6:94). The second and third solvent systems were used for the separation of different lipid classes to demonstrate a possible incorporation of the label into these lipids (*22*). The bands of the reference substances were detected and marked under UV light.

The activity on the plates was counted with a Berthold Multi-Tracemaster system, activity peaks were marked, and the relative distribution of activity on the plates between the different lipid fractions and the short-chain metabolites were calculated.

In Vivo Rat Hearts. 2-[5-(4-chlorophenyl)pentyl]-oxirane-2-carboxylate (POCA, obtained from Byk-Gulden Germany, Konstanz, FRG) inhibits carnitine-acyl transferase (*23,24*).

Male Wistar rats weighing 200–300 g were studied with this compound after 24 hr of fasting. Twenty minutes after induction of pentobarbital anesthesia, POCA (30 mg/kg body weight) was injected intraperitoneally. Controls received saline. Two hours later, 2.6–2.9 MBq of oPPA or pPPA were injected into the jugular vein. For each time interval (1, 2, 3, 4, 5, 7, 10, and 20 min) five animals were studied; their hearts were removed and immediately frozen in liquid nitrogen. At the same time, blood samples were taken from the inferior vena cava. The frozen hearts were weighed, homogenized, and extracted with chloroform-methanol (*21*), as described above. This provided fractions of total lipids, aqueous phase, and solid residue. Radioactivity in each fraction was counted and calculated as relative units or as a percentage of administered dose. The percent myocardial uptake was related to a standardized heart weight of 1 g.

Total lipids were further separated by TLC in parallel with standard lipid substances, such as phospholipids, cholesterol, cholesterol esters, tripalmitate, PA, oPPA, and pPPA (*13*).

In Vitro Test for Binding of Fatty Acids to CoA

oPPA, pPPA, PA, and IHA were dissolved in triton-X-100 (300 mg/l) in triplicates. The extent of activation of these fatty acids by acyl-CoA synthetase was analysed by a colorimetric enzymatic assay (Boehringer Mannheim, GmbH, Mannheim, FRG) for determination of free fatty acids (*25*). At 30, 60, 90, and 120 min of incubation of each solution, the generation of enoyl-CoA from acyl-CoA in the presence of peroxidase, with concomitant appearance of a red dye, was measured in the visible range at 546 nm.

Human Studies with Double-Labeled oPPA and pPPA

Intravenous Injection (Experimental Protocol). Eight patients (4 oPPA, 4 pPPA) with low probability of coronary heart disease were examined with informed consent. After simultaneous i.v. administration of 185 kBq pPPA or oPPA labeled with carbon-14 (^{14}C) and 100 MBq of pPPA or oPPA labeled with ^{123}I, respectively, a dynamic planar study of 60 min was acquired with the gamma camera in the anterior position. At 0, 3, 5, 10, 20, 30, 40, 50, 60, and 70 min, venous blood samples were taken.The blood samples were analysed for ^{123}I-labeled water-soluble compounds and ^{123}I-labeled compounds soluble in organic solvents. In addition, the exhaled air was measured, and both respiratory volume/minute and specific activity of exhaled $^{14}CO_2$ were determined.

Analysis of Blood Samples, ^{123}I-Labeled Compounds (Analytical Procedure). Blood samples (5 ml each) were taken from a peripheral vein with heparinized syringes. Two milliliters of serum were taken and extracted with chloroform-methanol, as described above (*21*).

The organic and water-soluble phases were separated and the paper filters, containing the solid residue of the serum, were counted for ^{123}I with a gamma scintillation counter. The activity of the phases was expressed in percent of the injected dose (%ID).

Analysis of Exhaled Air: $^{14}CO_2$. The exhaled air was led through a two-way-breathing mask and a tube to a gas meter for determining the respiratory volume/minute. At 0, 2, 5, 10, 15, 20, 30, 40, 45, and 50 min postinjection, a defined part of the exhaled air was led through a vial filled with a defined amount of methylbenzethonium hydroxide (Hyamine) (*26*); an indicator changed color when CO_2-saturation (1 mmol) was reached. After adding the scintillator solution, Insta-Gel (Canberra-Packard, Frankfurt/Main, FRG), the $^{14}CO_2$ activity was counted. The specific ^{14}C activity of the exhaled air was calculated as Bq/mmol CO_2.

Intracoronary Injection (Experimental Protocol). For investigating the human myocardium exclusive of other organs, another study was designed. Seven patients were examined (3 with oPPA and 4 with pPPA) after obtaining informed consent concerning the procedure. All patients underwent coronary angiography because of valvular heart disease. They had no apparent coronary heart disease. In addition to the left heart catheterization, another catheter was positioned in the venous sinus from the right atrium. oPPA or pPPA labeled with ^{14}C and ^{131}I (37 kBq for both) as described above were injected directly into the left coronary artery. Blood samples, of 5 ml each, were taken simultaneously at 0, 1, 2, 3.5, 5, 7, 10, and 15 min postinjection with heparinized syringes from the coronary sinus and the aorta. The latter samples were obtained for the correction for recirculating labeled catabolites.

The blood samples were analyzed for uncatabolized ^{14}C-labeled fatty acids, $^{14}CO_2$, uncatabolized ^{131}I-labeled fatty acids, and ^{131}I-labeled catabolites, i.e., ^{131}I-benzoic acid and ^{131}I-phenylpropionic acid. In the case of one patient, who received pPPA, only the ^{14}C-data were available.

Analytical Procedure: $^{14}CO_2$. The $^{14}CO_2$ was collected directly by a diffusion method (*27*). Two milliliters of each blood sample were placed in the outer well of a double-chambered glass-flask, especially designed for that purpose. The samples were slightly acidified with lactic acid and the tightly closed flasks were agitated on a shaker table for 24 hr. The center well of the flask was filled with 3 ml of 1 *M* Hyamine that trapped the released $^{14}CO_2$. In our laboratory, the recovery of $^{14}CO_2$ from $NaH^{14}CO_3$ added to whole blood in vitro by this method is more than 98%. The Hyamine solution with the trapped $^{14}CO_2$ was mixed with the scintillator Insta-Gel and counted.

Carbon-14-Labeled Fatty Acid. The ^{14}C-labeled fatty acid was determined by counting the ^{14}C-activity of serum samples, which had been freed from $^{14}CO_2$ by addition of lactic acid and agitation on a shaker table for 24 hr. The count rates of the serum were corrected for the ^{131}I-activity of these samples.

Iodine-131-Labeled Compounds. Two aliquots of 200 μl serum of each sample were counted in a gamma-scintillation counter for determination of total ^{131}I activity. The distribution of the label between catabolites and non-catabolised fatty acids was determined by TLC. For this purpose, the remaining serum of each sample was extracted with chloroform-methanol (*21*). The extracts were filtered, evaporated to dryness, and redissolved with a small volume of solvent, as described above.

Probes of each sample were separated by TLC, including chromatography of unlabeled reference substances, as described above.

The activity on the plates was counted with a Berthold Multi-Tracemaster system; the regions of interest were determined by the corresponding reference substances, and the relative distribution of activity on the plates between different lipid fractions and ^{131}I-labeled catabolites was calculated.

Activity for $^{14}CO_2$, ^{14}C-fatty acid, ^{131}I-catabolite, and ^{131}I-fatty acid was expressed in cpm/ml whole blood. When serum was analyzed, the results were corrected with the hematocrit.

Welch's t-test was applied throughout for determination of statistical significance of difference of means.

RESULTS

Animal Studies

In Vitro Rat Heart Perfusion. The total amount of ^{131}I-benzoic acid, ^{131}I-phenylpropionic acid, and ^{131}I-phenylated fatty acid as found in the effluates during a 30-min continuous infusion of pPPA and oPPA, expressed as percent of the infused activity, and the amount of the total activity retained in the hearts after the tracer infusion was analyzed (see Table 1).

Of the infused activity, 9.4% was taken up by the heart during pPPA infusion, 1.3% in the case of oPPA. In the effluate, 5.7% of infused pPPA and 0.2% of oPPA were found as iodo-benzoic acid, and 1.5% of pPPA and 0.3% of oPPA activity as iodo-phenylpropionic acid. The free fatty acids in the effluate amounted to 82.9% of infused pPPA and 97.7% of oPPA (Table 1).

Table 2 summarizes the activity distribution within the myocardium; the major difference between pPPA and oPPA is in the fraction of the free fatty-acids (only 1.0% for pPPA but 41.5% for oPPA). pPPA was mainly found esterified to phospholipids (34.3%) and triglycerides (49.5%). oPPA was esterified to a lower degree: 24.7% as phospholipids and 24.6% as triglycerides. The activity of short-chain catabolites in the myocardium was relatively low for both pPPA and oPPA, which indicates a rapid elimination of these catabolites into the effluates.

Figure 1 shows iodo-benzoic acid in the effluates in

TABLE 1
Total Activity Distribution in the Effluates and Total Heart in Isolated Perfused Rat Heart Study

	pPPA	oPPA	Sign. test (p≤)
Benzoic acid in effluates	5.7 ± 1.3	0.2 ± 0.0	0.01
Phenyl-Propionic acid in effluates	1.5 ± 0.3	0.3 ± 0.1	0.01
Free fatty acid in effluates	82.2 ± 1.6	97.7 ± 0.3	0.001
Heart activity	9.4 ± 0.6	1.3 ± 0.2	0.0001

TABLE 2
Activity in Different Lipid Fractions and Benzoic Acid in Isolated Perfused Rat Heart Study
(% Heart Activity, mean ± s.d., n = 4)

	pPPA	oPPA	Sign. test (p≤)
Phospholipids	34.3 ± 3.5	24.7 ± 1.0	0.01
Benzoic acid	3.5 ± 2.2	2.3 ± 0.2	n.s.
Phenyl-Propionic acid	0.1 ± 0.1	0.5 ± 0.6	n.s.
Free fatty acid	1.0 ± 0.2	41.5 ± 1.0	0.0001
Diglycerides	1.1 ± 0.4	0.0 ± 0.0	0.02
Triglycerides	49.5 ± 2.5	24.6 ± 0.7	0.0001
Solid residue	9.8 ± 1.1	6.4 ± 0.8	0.01

percent of the total effluate activity during the time of infusion. In the case of pPPA, up to 8.4% was eventually found as iodo-benzoic acid. In contrast, during oPPA infusion only little, if any, iodo-benzoic acid appeared. Similar results were obtained for iodo-phenylpropionic acid: a maximum activity of 2% and 0.44% of the effluate activity for pPPA and oPPA respectively. Therefore, there was little catabolism of oPPA.

In Vivo Rat Hearts. The myocardial uptake of pPPA (Fig. 2) showed a peak of 3.6% of injected dose per gram of tissue at 3 min with activity decreasing rapidly to 0.9% per gram of tissue at 20 min. oPPA had a lower peak of 2.8% per gram of tissue after 1 min and also rapidly decreased to 0.5% per gram of tissue at 20 min. After POCA treatment, pPPA showed progressively increasing uptake and retention within the myocardium and reached a plateau (3.5%) at 10 to 20 min. oPPA kinetics after POCA treatment, however, was only slightly different compared to the control group.

As shown in Table 3, after pPPA, normal rat hearts showed a maximum of ^{131}I activity of 0.93% of injected dose in the total lipid fraction and 0.66% in the water-soluble catabolites at 3 min. In the case of oPPA, the tracer was found mainly in the total lipids while activity in the water-soluble phase did not exceed a level of 0.16% during the time of observation. After POCA pretreatment, the activity of the water-soluble phase was lower than that in control hearts for pPPA throughout the study, and to a lesser degree for oPPA at times later than 4 min.

Table 4A gives the data of the chromatographic analysis of the total lipids; in the control group after pPPA, there was a rapid activity decrease of the free fatty acid fraction to undetectable amounts after 1 min. The main part of tracer activity was initially found in the triglycerides and phospholipids. In the case of oPPA, the activity was mainly in the unesterified free fatty acids during the time of observation.

In animals pretreated with POCA (see Table 4B), pPPA was mainly esterified to triglycerides, and free fatty acids could now be detected up to 10 min in significantly larger amounts compared to controls. In the case of oPPA, POCA slightly diminished the low tracer incorporation into complex lipids and increased the tracer in the free fatty acid pool.

In Vitro Test for Binding of Fatty Acids to CoA

The results of this enzyme test are listed in Table 5. PA and IHA showed rather constant high levels of activation (~90% for both) during the two hours of observation. The rates of activation of the phenyl fatty acids were much lower at the beginning of incubation and increased with time for pPPA from 48% to 81% and for oPPA from 32% to 62%.

Human Studies with Double-Labeled oPPA and pPPA

Intravenous Injection. The total radioiodine activity and the distribution between lipids and water-soluble fractions of blood serum after i.v. injection of double-

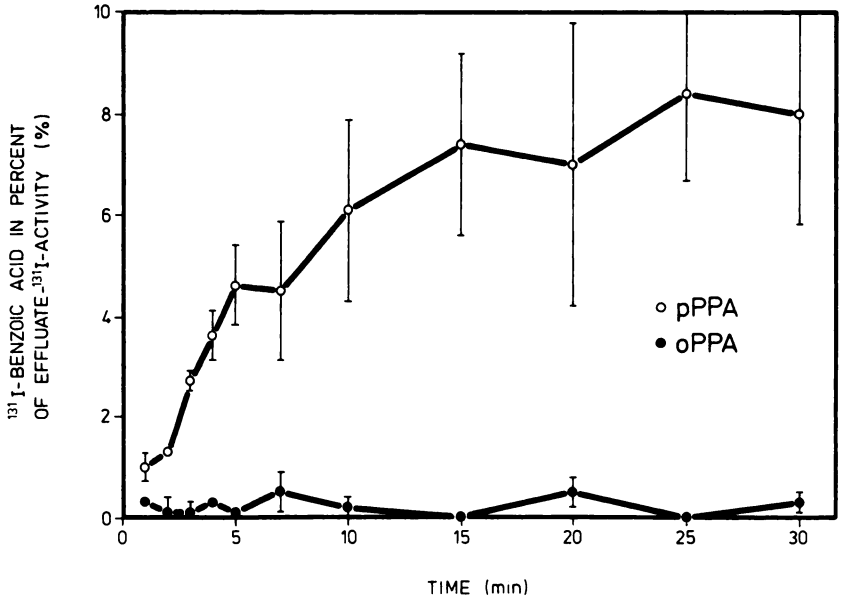

FIGURE 1
Iodine-131-benzoic acid in percent of effluate ^{131}I activity after continuous infusion of pPPA or oPPA in isolated perfused rat hearts (n = 4).

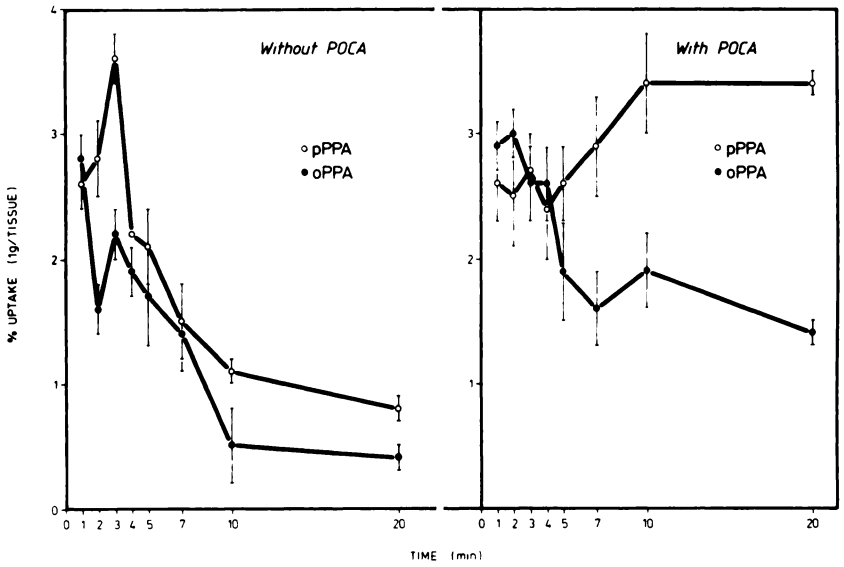

FIGURE 2
Total heart activity after i.v. injection of ^{131}I-labeled pPPA or oPPA in rats with and without pretreatment with POCA (phenylalkyl-oxyrane-carboxylic acid), which inhibits the carnitine fatty acid shuttle from the cytosol into mitochondria.

labeled pPPA and oPPA, expressed as %ID are shown in Figure 3.

In the case of pPPA, an early rise of water-soluble iodine catabolites could be observed. With oPPA, a significantly lower amount of iodine catabolites appeared.

Figure 4 (upper panel) shows the specific activity of $^{14}CO_2$ exhaled in air after intravenous injection. The rate of appearance of $^{14}CO_2$ after oPPA was significantly less than after pPPA from the tenth minute after injection. The fraction of water-soluble ^{123}I-catabolites in serum after oPPA was significantly smaller than in the case of pPPA, as shown in Figure 4 (lower panel).

Intracoronary Injection. Figure 5 shows the appearance of total ^{131}I- and ^{14}C-activity in the coronary sinus, expressed as %ID/ml. There is a significant difference between the curves for pPPA and oPPA indicative of a lower initial extraction of oPPA by the myocardium.

The distribution of the ^{131}I- and the ^{14}C-labeled catabolites in the coronary sinus blood is shown in Figure 6. In the case of pPPA, the ^{131}I-catabolites reached ~90% of the total activity of the samples 5 min after injection, while for oPPA a maximum of 46.5% for ^{131}I-catabolites was only reached after 15 min.

After pPPA injection, $^{14}CO_2$ reached 68.3% after 7 min and 84% after 15 min, while only 22.3% of the ^{14}C-activity were found as $^{14}CO_2$ in the case of oPPA after 15 min. For both pPPA and oPPA, $^{14}CO_2$ appeared later than the ^{131}I-catabolites.

In all, significantly less ^{131}I-catabolites and $^{14}CO_2$ were produced in parallel, after oPPA injection, compared to pPPA.

DISCUSSION

oPPA is readily taken up and retained in the human myocardium, as seen in dynamic gamma-camera studies with ^{123}I-labeled oPPA (Fig. 7) (*14,15*).

oPPA- and pPPA-catabolites that were measured in the peripheral blood after i.v. injection could be produced by the liver or other organs with a high lipid turnover, e.g., skeletal muscle. Still, measurement of serum activity distribution following i.v. injection of both tracers revealed a significantly higher concentration of pPPA-catabolites compared to oPPA.

TABLE 3
Water-soluble Activity after pPPA and oPPA in POCA Pretreated and Control Rats: In Vivo Heart Study

[% ID/g tissue, mean ± s.d., (n = 5)]					Significance test (p≤)			
Time	Control Animals		POCA Animals		Contr. vs. POCA		pPPA vs. oPPA	
(min)	pPPA	oPPA	pPPA	oPPA	pPPA	oPPA	Controls	POCA
1	0.32 ± 0.1	0.03 ± 0.01	0.12 ± 0.04	0.05 ± 0.02	0.01	n.s.	0.01	0.02
2	0.47 ± 0.2	0.05 ± 0.02	0.10 ± 0.02	0.09 ± 0.03	0.02	0.05	0.01	n.s.
3	0.66 ± 0.2	0.08 ± 0.03	0.12 ± 0.04	0.05 ± 0.02	0.01	n.s.	0.01	0.02
4	0.28 ± 0.2	0.04 ± 0.02	0.09 ± 0.02	0.04 ± 0.01	n.s.	n.s.	n.s.	0.01
5	0.33 ± 0.1	0.05 ± 0.03	0.10 ± 0.01	0.02 ± 0.01	0.01	n.s.	0.002	0.0001
7	0.32 ± 0.1	0.16 ± 0.1	0.06 ± 0.02	0.02 ± 0.00	0.01	0.05	0.05	0.02
10	0.21 ± 0.1	0.05 ± 0.01	0.05 ± 0.02	0.01 ± 0.00	0.05	0.001	0.05	0.02
20	0.11 ± 0.03	0.03 ± 0.01	0.06 ± 0.01	0.01 ± 0.00	0.05	0.01	0.01	0.001

TABLE 4A
Activity in Different Lipid Fractions after pPPA and oPPA in Control Rats: In Vivo Heart Study
(% ID/g Tissue, mean ± s.d., n = 5)

Time (min)	Free Fatty Acids		Triglycerides		Phospholipids	
	pPPA	oPPA	pPPA	oPPA	pPPA	oPPA
1	0.18 ± 0.001	1.57 ± 0.12	0.35 ± 0.02	0.0 ± 0.0	0.30 ± 0.01	0.0 ± 0.0
2	0.0 ± 0.0	1.11 ± 0.09	0.55 ± 0.03	0.0 ± 0.0	0.21 ± 0.01	0.0 ± 0.0
3	0.0 ± 0.0	0.89 ± 0.06	0.23 ± 0.01	0.02 ± 0.005	0.30 ± 0.01	0.07 ± 0.005
4	0.0 ± 0.0	0.77 ± 0.05	0.41 ± 0.02	0.05 ± 0.01	0.08 ± 0.003	0.03 ± 0.002
5	0.0 ± 0.0	0.63 ± 0.03	0.40 ± 0.02	0.14 ± 0.01	0.07 ± 0.003	0.03 ± 0.003
7	0.0 ± 0.0	0.49 ± 0.02	0.39 ± 0.02	0.13 ± 0.02	0.08 ± 0.004	0.17 ± 0.01
10	0.0 ± 0.0	0.23 ± 0.01	0.46 ± 0.03	0.05 ± 0.01	0.08 ± 0.003	0.02 ± 0.002
20	0.0 ± 0.0	0.10 ± 0.001	0.38 ± 0.01	0.13 ± 0.01	0.07 ± 0.004	0.03 ± 0.002

The special experimental design of intracoronary tracer application in humans allowed a correction for recirculating labeled catabolites.

After oPPA intracoronary injection, a small production rate of ^{131}I-labeled catabolites, i.e., iodo-benzoic acid and iodo-phenylpropionic acid, and of $^{14}CO_2$ was found. In contrast, a significantly higher production rate of catabolites was seen after pPPA injection. If ^{131}I-labeled fragments of oPPA had been retained in mitochondria, a higher rate of $^{14}CO_2$-production compared with that of ^{131}I-catabolites would have been expected. However, with both fatty acids a similar ratio of ^{131}I-catabolites and $^{14}CO_2$ was found. Therefore, the slow elimination of oPPA from the human myocardial cell is caused by a retention of the entire fatty acid in the cytosolic lipid pool. Moreover, the delayed appearance of $^{14}CO_2$ in the coronary sinus following intracoronary artery injection of pPPA and oPPA in comparison with ^{131}I-catabolites points to some intracellular reutilization of $^{14}CO_2$ or rapid transport of ^{131}I-labeled catabolites from the mitochondria into the circulating blood.

There is a species-related difference in the myocardial kinetics of oPPA; rat heart does not show a prolonged retention, in contrast to the human heart. This appears to be due to the inhibition of esterification to complex lipids and a high rate of backdiffusion of unchanged oPPA in rats. The animal studies using POCA help to better understand the phenomena. Inhibition of a part of the enzyme system carrying fatty acids into the mitochondria, i.e., of the carnitine-acyl-transferase I (CPT I), with POCA, a specific CPT I-inhibitor, altered the metabolic behavior of oPPA only slightly as compared to pPPA. In fact, there was a further slight diminution of oPPA esterification into complex lipids. On the other hand, in the case of pPPA, CPT I-inhibition resulted in a significant change of kinetics with a diminished β-oxidation and an augmented storage in the cytosolic lipid pool, mainly in triglycerides. The relatively rapid turnover of oPPA in the rat heart after POCA injection is likely due to backdiffusion from the myocardium into the vascular system.

In the continuously perfused rat heart, pPPA was rapidly catabolized, whereas oPPA was less well retained, again probably because of enhanced backdiffusion and lower rate of esterification to phospholipids and triglycerides.

The kinetic difference between pPPA and oPPA might be due to a change of the substrate conformation by the ortho iodine substitution on the phenyl ring (*28*). This has in principle also been observed for other substituted benzoic acid derivatives (*29*).

In accordance with these observations, the catabolism of oPPA by β-oxidation was shown to be highly reduced, probably because oPPA might be blocked from

TABLE 4B
Activity in Different Lipid Fractions after pPPA and oPPA in POCA Pretreated Rats: In Vivo Heart Study
(% ID/g Tissue, mean ± s.d., n = 5)

Time (min)	Free Fatty Acids		Triglycerides		Phospholipids	
	pPPA	oPPA	pPPA	oPPA	pPPA	oPPA
1	0.72 ± 0.05	1.44 ± 0.01	0.29 ± 0.01	0.0 ± 0.0	0.24 ± 0.01	0.0 ± 0.0
2	0.36 ± 0.02	1.41 ± 0.01	0.64 ± 0.04	0.0 ± 0.0	0.15 ± 0.01	0.0 ± 0.0
3	0.22 ± 0.01	1.25 ± 0.01	0.87 ± 0.06	0.0 ± 0.0	0.11 ± 0.01	0.0 ± 0.0
4	0.16 ± 0.01	1.17 ± 0.01	0.89 ± 0.06	0.0 ± 0.0	0.10 ± 0.01	0.0 ± 0.0
5	0.14 ± 0.01	0.91 ± 0.008	1.08 ± 0.09	0.02 ± 0.001	0.11 ± 0.01	0.0 ± 0.0
7	0.11 ± 0.01	0.76 ± 0.007	1.25 ± 0.11	0.02 ± 0.001	0.15 ± 0.01	0.0 ± 0.0
10	0.06 ± 0.001	0.68 ± 0.005	1.14 ± 0.09	0.07 ± 0.002	0.07 ± 0.003	0.0 ± 0.0
20	0.0 ± 0.0	0.33 ± 0.002	1.62 ± 0.12	0.10 ± 0.01	0.0 ± 0.0	0.0 ± 0.0

TABLE 5
In Vitro Test for Binding of Different Fatty Acids to CoA
(Percent of Binding to CoA, mean ± s.d.)

Time of incubation (min)	PA*	IHA†	pPPA‡	oPPA§
30	90.5 ± 3.5	83.2 ± 2.2	47.5 ± 2.2	31.7 ± 2.5
60	89.2 ± 2.5	87.0 ± 2.0	58.4 ± 2.4	43.2 ± 3.1
90	89.2 ± 2.4	87.2 ± 2.5	75.3 ± 2.3	57.2 ± 2.8
120	90.5 ± 3.6	90.8 ± 2.7	80.8 ± 2.5	61.7 ± 2.8

* PA = palmitic acid.
† IHA = 17-I-Heptadecanoic acid.
‡ pPPA = 15-(para-I-Phenyl)-pentadecanoic acid.
§ oPPA = 15-(ortho-I-Phenyl)-pentadecanoic acid.

binding with the carnitine-acyl-transferase, the carrier enzyme of fatty acids into the mitochondria.

A second important finding of our in vitro rat heart perfusion study is that only small amounts of the total infused oPPA and pPPA activity were found in the heart as short chain catabolites, indicating that in the rat the catabolites of these phenylated fatty acids are not retained in the myocardium. As this is probably also true in humans, the externally detectable activity after i.v. administration of oPPA and pPPA should not be influenced by retained labeled catabolites.

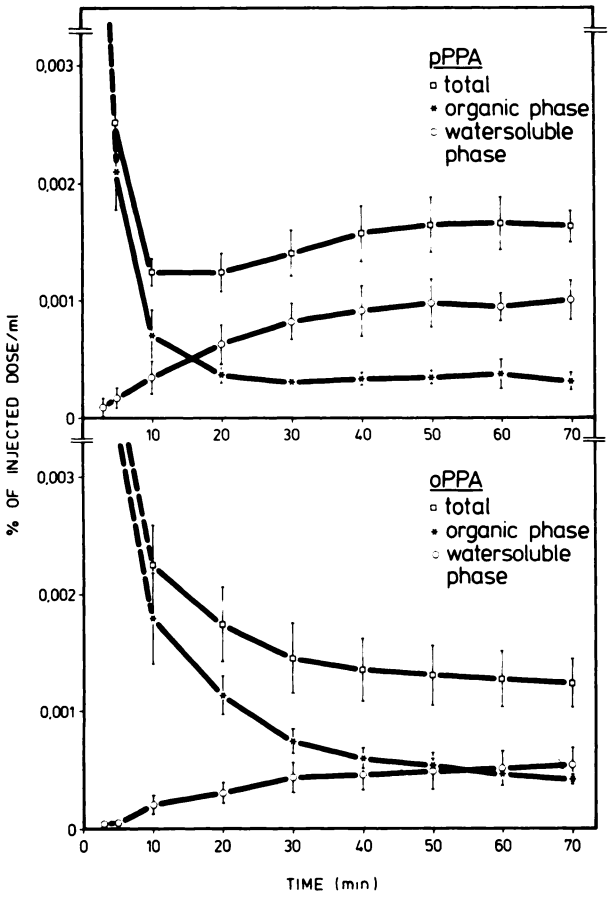

FIGURE 3
Iodine^{123} activity distribution in venous blood samples (serum) after i.v. injection of double-labeled pPPA (upper panel) or oPPA (lower panel) in man (n = 4). The samples were separated into organic (not catabolized pPPA or oPPA) and water-soluble phase (fatty acid catabolites).

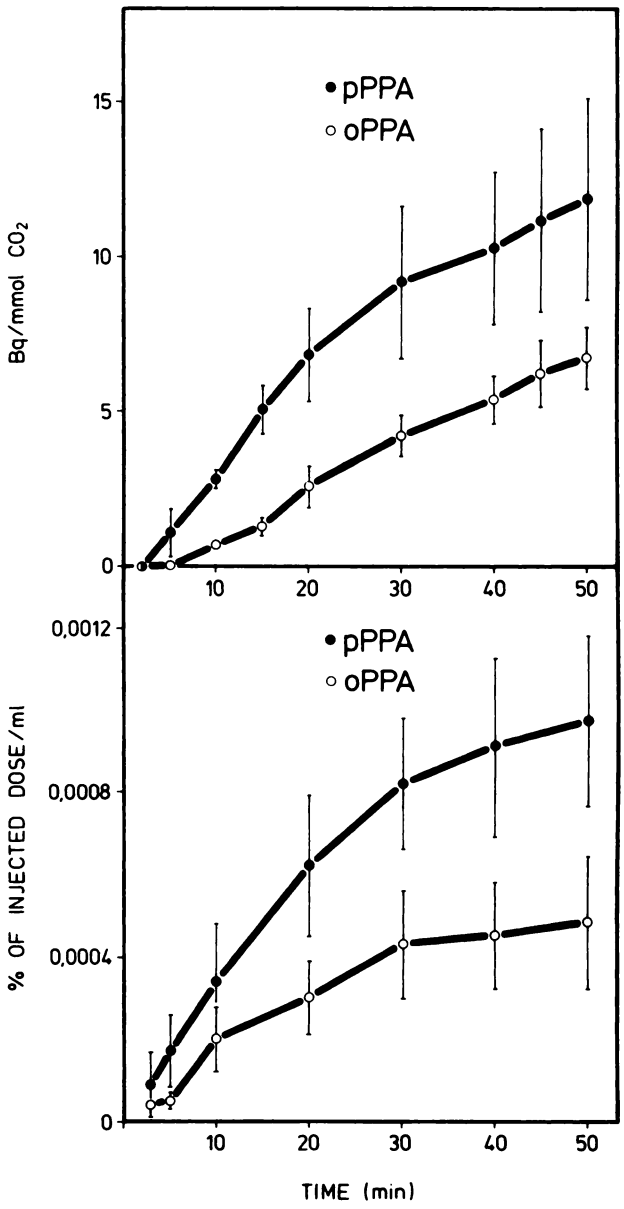

FIGURE 4
Specific activity of $^{14}CO_2$ in exhaled air (upper panel) and water-soluble ^{123}I-catabolites in venous blood samples (serum) (lower panel) after i.v. injection of double-labeled pPPA or oPPA in man (n = 4).

Another interesting detail of oPPA metabolism is given by the in vitro comparison of the activation of different fatty acids by acyl-CoA synthetase. pPPA and oPPA were not as quickly activated as IHA and PA. This data indicates that the phenylated fatty acids are to be used in man with caution if their metabolic uptake into the myocardium is to be studied.

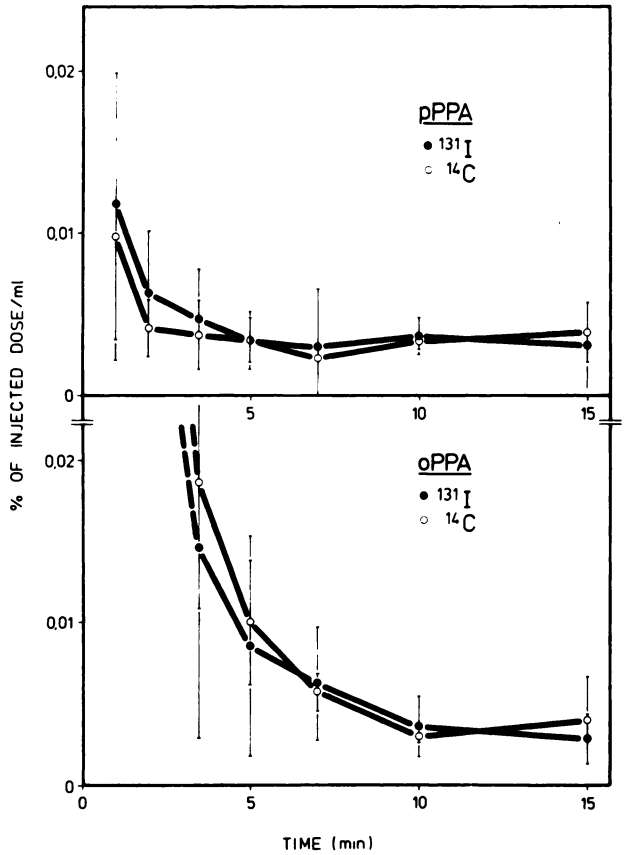

FIGURE 5
Total ^{131}I and ^{14}C activity in blood samples taken from the coronary sinus after intracoronary injection of double-labeled pPPA (upper panel) and oPPA (lower panel) in man (n = 3, ^{14}C-values of pPPA n = 4).

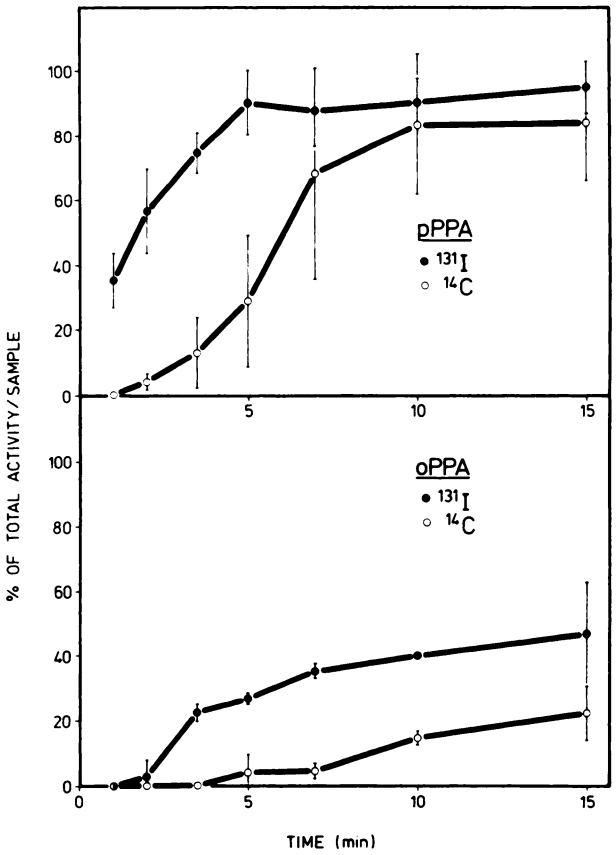

FIGURE 6
Iodine-131-labeled catabolites and $^{14}CO_2$ in blood samples taken from the coronary sinus after intracoronary injection of double-labeled pPPA (upper panel) and oPPA (lower panel) in man (n = 3, ^{14}C-values of pPPA n = 4).

The present data show that once oPPA and pPPA are incorporated into the metabolic chain, their retentions depend on specific subsequent metabolic reactions. Since the kinetics of oPPA deviates from that of pPPA, PA or IHA, clearly at the carnithine shuttle, resulting in trapping in the lipid pool in man, the externally measured difference between respective turnover curves may be exploited for probing the carnitine shuttle.

In conclusion, oPPA uptake in human myocardium seems to depend on local perfusion, regional myocardial free fatty acid extraction, and on a retention in the cytosol after coenzyme-A-activation. Irrespective of the uncertainty of the mode of this retention in the cytosol, oPPA deserves special interest for in vivo myocardial studies, because oPPA uptake not only depends on myocardial perfusion, but also serves as an indicator of the early steps of fatty acid metabolism in the myocardium.

The long retention of oPPA in the human myocardium is advantageous for time-consuming SPECT studies, where the image quality is aided by the ideal gamma-energy of ^{123}I (159 keV).

Most important, the use of oPPA in conjunction with a suitably labeled fatty acid analog that is not trapped in the cytosol but undergoes β-oxidation, like pPPA or IHA, i.e., an *in vivo* dual-tracer analysis, has the potential of evaluating fatty acid transport into

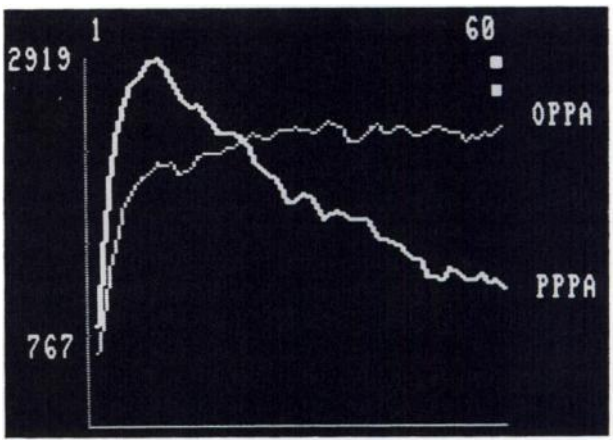

FIGURE 7
Time-activity curves from human myocardium after i.v. injection of 140 MBq ^{123}I-labeled pPPA or oPPA from dynamic studies over 60 min, 2 frames/min, in anterior position without zoom. (Y-axis: counts/30 sec).

mitochondria, i.e., the function of the carnitine shuttle, which may be of considerable clinical interest.

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