Myocardial Metabolism of Radioiodinated Methyl-Branched Fatty Acids

Luc Demaison, Francis Dubois, Marcel Apparu, Jean-Paul Mathieu, Michel Vidal, Michel Comet, and Pierre Cuchet

University of Sciences, Technology and Medicine, Grenoble, France

Methylated fatty acids labeled with radioactive iodine have been proposed as a means of studying regional myocardial uptake of fatty acids in man. To investigate the methylated fatty acid that is best adapted for an assessment of uptake, we have studied the influence of the number and the position of the methyl groups on IFA intracellular metabolism; 16-iodo-2-methyl-hexadecanoic (mono-alpha), 16-iodo-2,2-methyl hexadecanoic (di-alpha), 16-iodo-3-methyl-hexadecanoic (mono-beta), and 16-iodo-3,3-methyl-hexadecanoic (di-beta) acids were injected into the coronary arteries of isolated rat hearts. Intracellular analysis shows that the degradation of mono-alpha was always lower than that of IHA and the storage was always much higher. The differences between mono-beta and IHA were similar to those observed with mono-alpha, but were much more pronounced. With the two dimethylated IFAs there was an inhibition of both oxidation and esterification which led to an accumulation of free FAs in myocardial cells. In conclusion, mono-beta, di-alpha, and di-beta are potentially suitable for studying the cellular uptake of IFA since all of them, and particularly the dimethylated IFAs, have a low oxidation rate.

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Radioiodinated fatty acids (IFA) have been proposed as a means of studying myocardial metabolism by external detection in man (*1,2*) but their rapid mitochondrial degradation makes it difficult to evaluate their regional uptake using single photon emission computed tomography (SPECT). Iodomethylated FAs have been proposed for selective evaluations of regional FA uptake (*3,4*). To investigate the influence of the number and position of the methyl groups on IFA intracellular metabolism, we injected different methylated FAs labeled with radioactive iodine into the coronary arteries of isolated rat hearts perfused according to the method of Langendorff. At different postinjection times, the hearts were homogenized and the time course of radioactivity was measured in the different fractions of the cellular extract. The results were analyzed with a compartmental mathematic model of the intramyocardial fate of IFA.

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For reprints contact: Pierre Cuchet, Laboratory of Animal Physiology, BP 68, Domaine Universitaire, 38402 Saint Martin D'Heres Cedex, France.

MATERIALS AND METHODS

Fatty Acids

The following methylated IFAs were used: 16-iodo-2-methyl hexadecanoic acid (mono-alpha); 16-iodo-2,2-methyl hexadecanoic acid (di-alpha); 16-iodo-3-methyl hexadecanoic acid (mono-beta); and 16-iodo-3,3-methyl hexadecanoic acid (di-beta). A nonmethylated IFA, 16-iodo-9-hexadecenoic acid (IHA) (*5*), was used for reference.

Intracellular Analysis

Male Wistar rats, weighing 250 to 300 g, were anesthetized by an intraperitoneal injection of 6% sodium pentobarbital. The heart was removed and perfused in an open system via the aorta (Langendorff technique) with a Krebs-Henseleit solution containing only glucose (11 m*M*) and insulin (10 IU/l) as energy substrates. The solution was saturated with carbogen (95% O_2, 5% CO_2) and kept at pH 7.4 and 37°C. The partial pressure of oxygen was 600 mmHg and the perfusion flow was maintained at 10 ml/min. Following a 5-min preperfusion, we administered a bolus injection of 0.2 ml of a solution of one of the iodine-123- (^{123}I) labeled FAs (3.5 MBq), bound to albumin (FA/albumin ratio of 2) into coronary arteries. Intracellular analysis was done 60, 150, 300, and 600 sec after the IFA injection. Following perfusion, the ventricles

were removed, washed in Krebs medium at 4°C and quickly clamped and preserved in liquid nitrogen. Lipid extraction from the tissue homogenate was done according to the Bligh and Dyer technique (*6*) at 4°C with 15 ml of a mixture of methanol/chloroform/water (25:10:8, v/v/v) containing an antioxidant (0.05% butylated hydroxytoluene). After a 30-sec homogenization with a polytron, 2 ml of chloroform and 1 ml of distilled water were added; after a further 30-sec homogenization, 5 ml of chloroform was added before a final treatment with the polytron. The aqueous phase, lipid phase, and cellular residue were separated by centrifugation (1600 g) for 20 min at 0°C. The radioactivity of each phase was determined directly. The radioactivity of the aqueous phase was in iodide form. The different classes of lipids were separated by thin layer chromatography (Kieselgel $60F_{254}$—Merck): neutral lipids were eluted in a mixture of petroleum ether/diethyl ether/acetic acid (85:15:2, v/v/v) and polar lipids in a mixture of chloroform/methanol/water (65:25:4, v/v/v). After migration, the lipids were revealed by iodine vapors; the silicagel bands corresponding to each category of lipid were scraped from the plate, the powder was collected and put into tubes, and radioactivity was measured with an NaI crystal counter. Results of intracellular analysis are expressed as percentages of total cardiac activity.

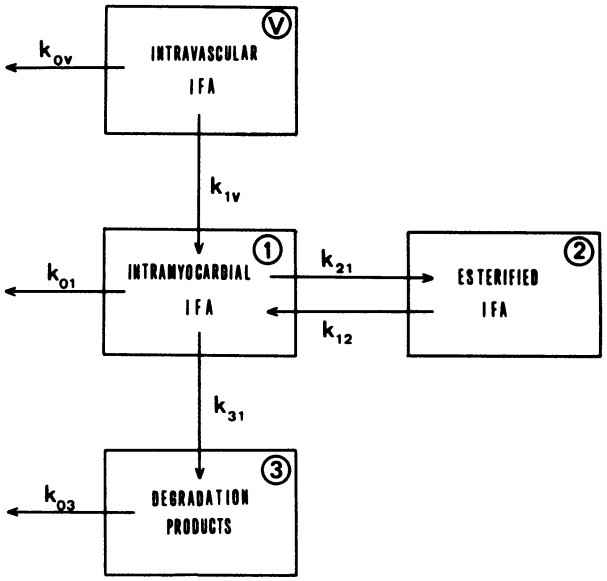

FIGURE 1
Compartmental mathematic model of the myocardial metabolism of iodinated fatty acids (IFA).

Mathematic model

Description

The model has four compartments (Fig. 1). Compartment v corresponds to intravascular IFA, Compartment 1 to intramyocardial free IFA (FIFA), Compartment 2 to the esterified forms of IFA, and Compartment 3 to the IFA degradation products. The rate constants characterizing the model signify the following: k_{0v} corresponds to the transit kinetics of IFA not taken up; k_{1v} to IFA uptake by myocardial cells; k_{01} to the backdiffusion of intramyocardial IFA into the capillaries; k_{21} to IFA esterification; k_{31} to the mitochondrial degradation of IFA, and k_{03} to the release of degradation products into the capillaries. The model is based on the following hypotheses (*7*): (a) IFA has zero or first-order kinetics; (b) the different rate constants do not change during measurement; (c) IFA that is taken up and backdiffused cannot be taken up again; (d) there is no intracellular synthesis of IFA from degradation products; and (e) IFA degradation products released into the capillaries cannot be taken up again.

Calculation Methods

The equations of the model are as follows:

$$q_v(t) = q_0 e^{-(k_{0v}+k_{1v})t}$$
$$q_1(t) = [k_{1v}q_v(t) + k_{12}q_2(t)]_* e^{-(k_{01}+k_{21}+k_{31})t}$$
$$q_2(t) = k_{21}q_1(t)_* e^{-k_{12}t}$$
$$q_3(t) = k_{31}q_1(t)_* e^{-k_{03}t},$$

where q_0 is the radioactivity injected per g of heart; $q_v(t)$, $q_1(t)$, $q_2(t)$ and $q_3(t)$ are the iodine activities per g of heart in compartments v, 1, 2, and 3; * is the convolution product.

The activities in the model compartments were fitted to the experimentally measured activities as follows: $q_1(t)$ was fitted to the activity of the free IFAs, the sum $\{q_1(t) + q_2(t)\}$ was fitted to the activity of the lipid phase, and $q_3(t)$ was fitted to the activity of the aqueous phase, which contained the iodinated degradation products of IFA. The three measured values were independent, i.e., an error in determining one of them had no influence on the others. The value of k_{0v} was obtained after injecting a bolus of ^{131}I-albumin into the coronary arteries of six isolated rat hearts under retrograde perfusion. The time course of cardiac radioactivity was measured by external detection (*8*). The descending portion of the time-activity curve was taken as an exponential with a slope of k_{0v}. To obtain the values of k_{01}, k_{1v}, k_{21}, k_{31}, k_{12} and k_{03} that gave the best fit between experimental and theoretical values, we used the Gauss-Newton algorithm with the least squares method for all three curves. The iterative process was stopped when the relative variation of the sum of the squares of the deviations between all the measured values and the theoretical values of the model fell below 0.01. To determine the dispersion of the rate-constant values, the calculation was repeated 25 times by randomly simulating the measured values of each point according to a Gaussian law centered on the mean value, with the standard deviation equal to the standard error of the mean (s.e.m.) measured at each point (*9*). We calculated the mean value and the standard deviation of the rate constants in each of the 25 calculations. Based on the calculated values of the different rate constants, the time courses of q_1, $(q_1 + q_2)$, and q_3 were plotted and graphically superimposed on the experimental time-activity curves.

Sensitivity Functions

To check the precision of the calculation of each rate constant, the following sensitivity functions were calculated (*10*):

$$\frac{\partial q_1(t)}{\partial k_{ij}} \quad \frac{\partial\{q_1(t) + q_2(t)\}}{\partial k_{ij}} \quad \frac{\partial q_3(t)}{\partial k_{ij}},$$

where $k_{ij} = k_{0v}, k_{12}, k_{01}, k_{21}, k_{31}, k_{03}$. We calculated the mean value of each sensitivity function for each rate constant over

all the measurement times. We then calculated the mean value of the three sensitivity functions. This value expresses the variation of the percentage of injected activity per g of heart per unit of k_{ij} variation. The higher the value of the sensitivity function of a rate constant, the more precisely it is determined, since a small change in its value leads to a large variation in the theoretical values of radioactivity. When the sensitivity function of a rate constant is low, it cannot be precisely determined and only its order of magnitude can be obtained.

Statistical Analysis

Student's t-test was applied to the data to determine the significance of the means.

RESULTS

Intracellular Analysis

Table 1 shows the values of radioactivity measured in the different cell fractions expressed as a percentage of total cardiac activity. The esterified forms (EIFA) included the diglycerides, triglycerides, and phospholipids. The activity in the aqueous phase represented the degradation products of IFA. With mono-alpha, the percentage of activity in the aqueous phase was always lower than with IHA, whereas the percentage of activity in EIFA was always much higher. The differences between mono-beta and IHA were similar to those observed with mono-alpha, but were much more pronounced. With di-alpha and especially di-beta, the percentage of activity in the aqueous phase was always very low whereas the percentage in the form of free IFA (FIFA) was always very high. Despite the abundance of free forms 30 sec postinjection, the percentage of activity in esterified forms was half of that observed with IHA. Thus, with the two dimethylated IFAs, there was an inhibition of both oxidation and esterification.

Table 2 shows the distribution of the different IFAs between polar lipids (acyl CoA, acyl carnitine, and phospholipids) and triglycerides, expressed as percentages of total myocardial activity. It can be seen that IHA was distributed nearly equally between triglycerides and polar lipids until 300 sec postinjection and was then preferentially stored in triglycerides at 600 sec postinjection. The activity of the monomethylated IFA was found essentially in the triglycerides at all times postinjection. With the dimethylated IFA, the percentage of activity was always larger in the triglycerides than in the polar lipids, but the values gradually increased between 60 and 600 sec postinjection, indicating a significant inhibition of esterification.

Mathematic Analysis

Sensitivity functions (*Table 3*). The results are expressed as percentages of injected activity per g of heart per min, i.e., each value represents the variation of the percentage of activity per g of heart for a rate-constant variation of one unit (Table 3). Consequently, as this value increases, the precision of the measurement of the rate constant becomes greater. The highest values appeared with the dimethylated IFAs. The determinations of mono-alpha and mono-beta were less precise than those of the dimethylated IFAs except with respect to K_{03}. IHA had the lowest values for the sensitivity functions, except again for K_{03}.

Rate constant values (*Tables 4 and 5*). The value of k_{0v} was 6.02 ± 1.25 per min. The rate constant values corresponding to lipolysis (k_{12}) and backdiffusion (k_{01}), were very low for all the IFAs. The rate constants corresponding to esterification (k_{21}) were much lower for the dimethylated IFAs than for the monomethylated IFAs and IHA. The rate constant corresponding to oxidation (k_{31}) was much higher for IHA than for the methylated IFAs.

Using the calculated values of the rate constants, the time courses of $q_1(t)$, $q_1(t) + q_2(t)$ and $q_3(t)$ were plotted and graphically superimposed on curves plotted from

TABLE 1
Time Course of Radioactivity, After a Bolus Injection of Iodinated Fatty Acids (IFA) Measured in the Free Forms (FIFA), Esterified Forms (EIFA) and Aqueous Phase (AQ) of the Extract*

Time postinjection	60 sec			150 sec			300 sec			600 sec		
	FIFA	EIFA	AQ	FIFA	EIFA	AQ	FIFA	EIFA	AQ	FIFA	EIFA	AQ
IHA	8.10	21.3	62.00	1.90	27.54	62.00	1.53	22.56	69.17	1.10	49.52	42.70
n = 5,5,5,5	±2.40	±4.20	±3.96	±0.50	±3.85	±1.40	±0.92	±2.99	±3.14	±0.40	±12.90	±13.00
Mono-alpha	13.39	60.56	13.11	1.06	64.34	23.43	1.71	66.80	18.91	1.74	75.37	12.14
n = 4,4,5,5	±6.35	±5.69	±0.88	±0.22	±6.57	±4.69	±0.24	±3.90	±2.38	±0.50	±2.93	±1.72
Mono-beta	43.44	37.59	7.45	5.85	72.86	8.94	4.91	78.59	4.70	3.02	77.33	7.27
n = 4,5,5,4	±1.72	±2.22	±0.43	±0.93	±3.88	±1.71	±0.81	±2.52	±1.19	±0.40	±1.93	±0.26
Di-alpha	74.39	12.87	6.93	56.02	29.43	3.63	29.50	56.78	5.15	12.27	74.81	10.07
n = 5,5,5,5	±3.05	±1.64	±1.08	±5.57	±5.76	±0.17	±9.19	±8.51	±0.74	±1.07	±1.52	±1.17
Di-beta	76.29	13.12	5.35	73.55	15.81	5.27	56.46	32.72	4.60	49.00	43.98	2.37
n = 4,4,5,5	±4.24	±3.82	±0.59	±1.32	±1.13	±0.33	±3.64	±3.89	±0.25	±5.78	±5.48	±0.60

* The values are expressed as percentages of total cardiac activity; n = the number of hearts analyzed at each time p.i.

TABLE 2
Distribution of Iodinated Fatty Acids (IFA) Between Polar Lipids and Triglycerides at Different Times After Injection of IFA*

Time postinjection	Polar lipids				Triglycerides			
	60 sec	150 sec	300 sec	600 sec	60 sec	150 sec	300 sec	600 sec
IHA	14.88	13.74	12.20	19.91	9.10	15.90	11.60	30.60
n = 5,5,5,5	±3.29	±2.75	±1.76	±6.28	±1.30	±1.60	±1.80	±8.50
Mono-alpha	18.43	15.08	11.34	14.34	42.11	49.26	51.25	61.02
n = 4,5,5,5	±2.11	±1.91	±3.87	±1.06	±3.86	±8.80	±6.55	±3.48
Mono-beta	8.37	15.36	20.80	18.15	29.22	57.48	57.79	59.18
n = 4,5,5,4	±1.67	±2.22	±6.58	±4.39	±1.74	±5.68	±8.43	±4.29
Di-alpha	5.87	2.04	3.07	10.00	6.97	27.39	50.33	64.8
n = 5,5,5,5	±0.28	±0.43	±0.34	±1.47	±1.41	±4.34	±3.88	±2.69
Di-beta	5.29	2.04	5.54	3.23	7.60	13.78	27.27	40.75
n = 4,4,5,5	±0.39	±0.30	±0.18	±0.29	±4.14	±1.35	±3.89	±5.56

* The values are expressed as percentages of the total myocardial activity; n = number of hearts studied at each time p.i.

the measurements of radioactivity in, respectively, the free FA, the lipid phase, and the aqueous phase of myocardial extracts (Fig. 2). It can be seen that the overall superimposition of the experimental and theoretical curves is quite satisfactory.

DISCUSSION

Regional myocardial uptake of fatty acids is not only related to coronary flow. It has been shown that when coronary flow decreases at a constant cardiac load, the myocardial uptake of fatty acids increases (*11*). The regional myocardial distribution of fatty acids is not always the same as that of thallium-201; this is the case in the hypertensive rat (*12*) and in regions reperfused after ischemia (*13*). Heterogeneous regional myocardial distribution of fatty acids has been demonstrated in man during cardiomyopathy (*14,15*). SPECT analysis appears to be required for evaluating this distribution and consequently the myocardial distribution of radioactivity must remain stable for 30 min postinjection. This is not the case after an injection of IHA. Its myocardial cellular uptake and metabolic distribution between the pathways of oxidation and storage are close to those of physiological FA (*8*). The iodine heteroatom is removed from the IHA molecule during beta-oxidation and released into the circulation. Because of its rapid cellular oxidation, IHA is not very suitable for studying the regional uptake of FA, and in man the corresponding myocardial radioactivity decreases by half in ~20 min.

TABLE 3
Values of Sensitivity Functions Expressed As Percentages of Activity per g of Heart per min

	k_{1v}	k_{21}	k_{31}	k_{03}	k_{12}	k_{01}
IHA	2.66	1.55	0.58	4.20	6.17	0.87
Mono-alpha	3.75	1.49	2.80	2.43	8.08	2.10
Mono-beta	3.32	3.40	5.53	0.81	7.72	4.16
Di-alpha	3.59	12.10	14.72	0.62	9.27	8.96
Di-beta	4.23	14.30	21.23	0.42	5.15	9.17

Since methylated FAs are catabolized more slowly than IHA, they have been proposed for studying the phase of FA cellular uptake (*3,4*). The presence of two methyl groups on the alpha carbon and one or two methyl groups on the beta carbon blocks the start of the molecule's beta-oxidation (*3,16*). By contrast, the presence of a single methyl group on the alpha carbon should not inhibit FA degradation. Maximum myocardial uptake of IFA is obtained with a chain length of 16 carbon atoms (*3*). Injected into the coronary arteries of perfused isolated rat hearts, mono-alpha, mono-beta and di-beta show a myocardial uptake equivalent to that of IHA (*17*). In the present study di-alpha and di-beta were oxidized very slowly, as indicated by the small proportion of intracellular iodides measured. Di-alpha appears to undergo omega-oxidation followed by incomplete beta-oxidation (*18*), whereas di-beta appears to undergo alpha-oxidation followed by beta-oxidation (*19,20*). The very small proportion of iodide found fixes an upper limit on the possible nonspecific deiodination, and makes it possible to confirm that the release of iodide after an injection of IFA only takes place during oxidative metabolism (*8*). The esterification of dimethylated IFAs was slower than that of IHA and appeared essentially in the form of triglycerides. Inhibition of esterification and weak oxidation of methylated FAs have previously been reported in rats fed with 2,2 dimethyl stearic acid (*18*). Consequently, dimethylated IFAs remain longer in the form of FIFA inside myocardial cells and this could be the cause of the rhythmic disturbances observed in isolated rat heart after the injection of di-beta (*17*).

Mono-alpha should theoretically undergo normal beta-oxidation. However, compared to IHA, this IFA

TABLE 4
Values of Rate Constants Expressed per min

IFA	Rate constant					
	k_{1v}	k_{21}	k_{31}	k_{03}	k_{12}	k_{01}
IHA	0.980	0.766	2.610	0.204	0.115	0.0338
	±0.024	±0.049	±0.142	±0.021	±0.0064	±0.0735
Mono-alpha	0.922	1.262	0.532	0.120	−0.0286	0.0105
	±0.047	±0.244	±0.069	±0.018	±0.0032	±0.033
Mono-beta	1.069	0.602	0.185	0.276	−0.032	0.0599
	±0.037	±0.020	±0.002	±0.002	±0.0065	±0.0089
Di-alpha	0.937	0.164	0.034	0.108	−0.032	0.0649
	±0.033	±0.020	±0.002	±0.002	±0.0065	±0.0081
Di-beta	0.840	0.133	0.0216	0.284	0.134	0.0089
	±0.036	±0.010	±0.0021	±0.008	±0.069	±0.0015

resulted in a smaller proportion of iodides and a larger proportion of esterified forms at the different postinjection times. This discreet inhibition of beta-oxidation with mono-alpha has previously been reported in rats fed with 2-methyl stearic acid (*21*). Mono-alpha was preferentially esterified in the form of triglycerides and its concentration in the form of FIFA was close to that of IHA.

Mono-beta was slowly oxidized. The existence of this degradation has been demonstrated using beta-methylated FA labeled with carbon-11 (^{11}C) (*22*), as well as in studies of Refsum's disease (*19,20*). Thus, the intracellular iodides are not the result of nonspecific deiodination, as suggested by certain authors (*3,23*). The proportion of FIFA observed with mono-beta was larger than that with IHA or mono-alpha. Esterification mainly took the form of triglycerides, as reported elsewhere (*24*).

To quantify the rates of IFA oxidation and esterification, we used a compartmental mathematical model of IFA myocardial metabolism. Unlike the mathematic model used to analyze the time-activity curves based on external detection after injection of IHA into the coronary arteries of an isolated rat heart (*7*), the model used here takes into account backdiffusion and lipolysis. The rate constants could be calculated with greater absolute precision when the kinetics were slower. Consequently, with IHA, and to a lesser extent with mono-alpha, we only essentially obtained the order of magnitude of the rate constants.

TABLE 5
Statistical Comparison of the Rate Constants*

k_{1v}	mono-beta > IHA; mono-alpha, di-alpha > di-beta
k_{21}	mono-alpha > IHA > mono-beta > di-alpha, di-beta
k_{31}	IHA > mono-alpha > mono-beta > di-alpha > di-beta
k_{03}	mono-beta, di-beta > IHA > mono-alpha, di-alpha
k_{12}	di-beta > IHA, mono-alpha, mono-beta, di-alpha
k_{01}	mono-beta > mono-alpha, di-alpha > di-beta (IHA not significantly different)

* ">" represents a significant difference ($p < 0.05$).

The rate of mono-alpha esterification was much higher than that of IHA and mono-beta, whereas that of the dimethylated IFAs was particularly low. The rate of mono-alpha oxidation was between those of IHA and mono-beta, whereas those of the dimethylated IFAs were very low. The rate constant for the backdiffusion of IFA was very low. This absence of backdiffusion despite the very high cellular concentrations of FIFA observed with the dimethylated IFAs was probably due to the experimental model used. The theoretical result is in agreement with studies on the effluents of isolated heart, in which the analysis showed no IFA after the passage of injected IFA that had not been taken up (*17*). By contrast, in vivo in the dog, the back-diffusion of ^{11}C beta methyl-heptadecanoic acid from myocardial cells toward the capillaries has been estimated to be 33% of the activity taken up (*25*). In our study, the rate constant for lipolysis was nearly zero for all the IFAs studied, except for di-beta. This result is only valid for the model used, since it has been shown that in isolated rat heart under load, more than half of the cardiac triglycerides are degraded after 30 min of perfusion, both with and without glucose in the perfusion liquid (*26*).

In conclusion, mono-beta, di-alpha and di-beta are potentially suitable for studying the cellular uptake of IFA, since all of them, particularly the dimethylated IFAs, have low oxidation rates. The absence of backdiffusion, the fact that the heart is not under a load, and the single transit of iodinated fatty acid through the coronary arteries represent important differences between the isolated rat heart and in vivo conditions. For these reasons, experimental studies in intact animals are needed before a methylated fatty acid can be chosen that is best adapted for studies in man.

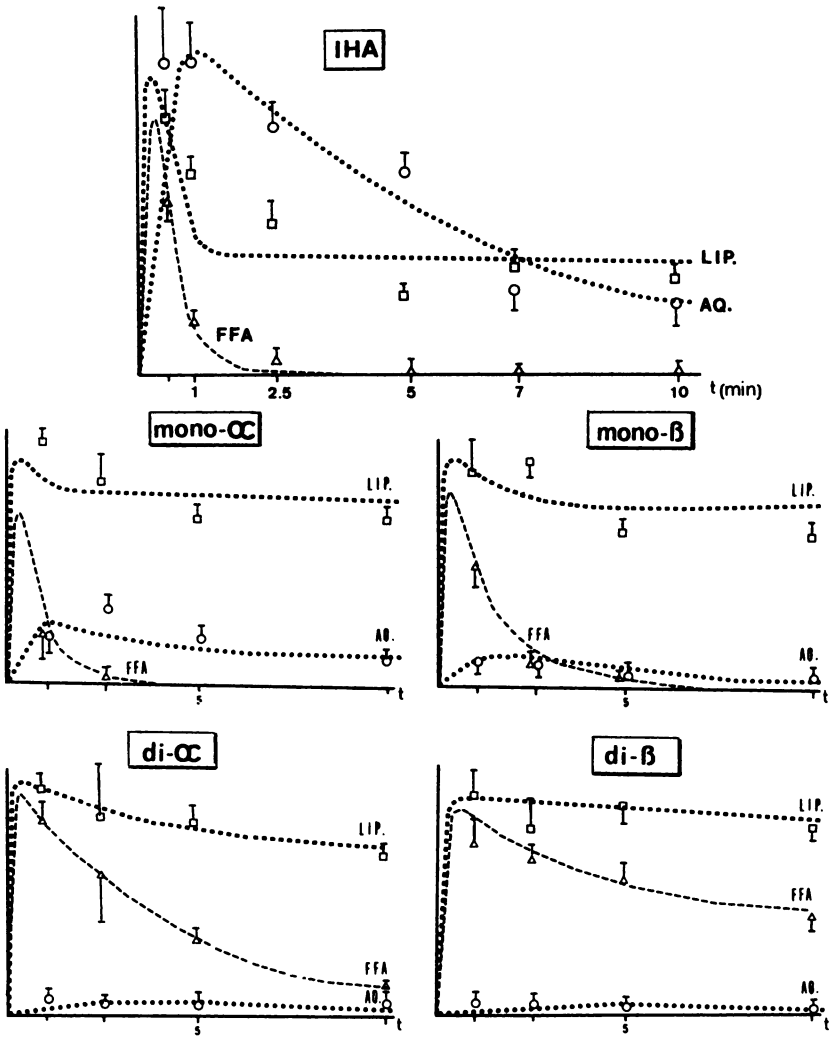

FIGURE 2
Superimposition of theoretical curves on experimental values of the time courses of radioactivity in the aqueous phase (AQ) lipid phase (LIP) and in free fatty acids (FFA). Symbols: [· · · ·; O] aqueous phase; [■ ■ ■ ■; □] lipid phase; [----; Δ] FFA; ⊥ s.e.m.

In vivo studies in the rat have been carried out with 15-(iodophenyl-p) pentadecanoic acid (IPP), 15-(iodophenyl-p)-3-R,S-methylpentadecanoic acid (BMIPP), and 15-(iodophenyl-p)-3,3-dimethylpentadecanoic acid (DMIPP) (*27*). The myocardial uptake of these fatty acids is comparable, but DMIPP shows the largest myocardial storage and the most rapid blood clearance. As in the case of mono-beta, di-alpha, and di-beta, the prolonged storage of BMIPP and DMIPP compared to that of IPP seems to be due to a cellular accumulation of the free (FIFA) and esterifies (EIFA) forms of iodinated fatty acid. Comparative studies of phenylated and non-phenylated fatty acids appear to be advisable.

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