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Metabolites of IPPA, BMIPP, and DMIPP Fatty Acids in Rat Hearts. A Quantitative HPLC-Study.

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INTRODUCTION

The significance of the use of radioiodinated fatty acid analogues such as 15-(p-iodophenyl)pentadecanoic acid (IPPA), 15-(p-iodophenyl)-3-R,S-methylpentadecanoic acid (BMIPP), and 15-(p-iodophenyl)-3-dimethylpentadecanoic acid (DMIPP) for the evaluation of regional myocardial fatty acid uptake is well documented. An understanding of the relative incorporation of these fatty acid analogues into various fatty acid pools is important to correlate fatty acid uptake and release with flux through the various metabolic pathways. While the free fatty acid (FFA) pool is immediately available for oxidation to meet energy demands for contraction under normoxic conditions, the FFA are also stored as triglycerides (TG) for oxidation at a later period.

Although the incorporation of IPPA, BMIPP, and DMIPP into the major phospholipid classes has not been previously investigated, demonstration of the incorporation of IPPA and BMIPP into phospholipids would be important as further evidence that these "modified" fatty acids are, at least qualitatively, metabolized through the usual metabolic pathways. The goals of the present studies were thus to develop the necessary HPLC techniques and evaluate the incorporation of IPPA, BMIPP, and DMIPP into complex endogenous lipids in dual label studies.

MATERIALS AND METHODS

Hearts were removed from female Fischer rats and quickly mounted by insertion of a blunt 18-gauge needle in the aortic root. Lines were inserted in the pulmonic artery and the left auricle for antegrade perfusion. Afterload was provided by a 1 m buffer column. The hearts were perfused at 8-10 ml/min in the usual manner with Krebs-Henseleit buffer at 37°C which contained albumin and lactate. A mixture of [I-125]BMIPP and [I-131]IPPA or [I-125]DMIPP and [I-131]IPPA in a small volume (100-150 microliter) of 6% delipidated BSA was quickly injected into a port. Blood gases (O_2 450-600 mm; CO_2 45-50 mm), perfusate pH (7.34-7.42) and the rate of contraction (180-210/min) were monitored throughout the studies and were within the normal ranges. After 15 min, the hearts were quickly dismounted, cooled and homogenized. Lipids were extracted by an acidified Folch-Technique, dried and frozen until analysis by HPLC.

For analysis the lipids were disssolved in 2 ml of chloroform/methanol 2:1 and filtered through a 0.45 µ filter.

For HPLC analysis a Beckman system with dual pumps attached to a PC were used. The tunable UV-detector and the gradient was controlled by a 406 interface. The chromatograms were analyzed by the software provided by Beckman. The endogenous phospholipids (PL) were detected by UV at 203 nm and non-polar lipids (NPL) at 214 nm. All lipid standards were obtained from Sigma Chemical Co. or Supelco, Inc. and were of 98-99% purity. All solvents were HPLC grade. The system was calibrated (peak area versus amount) with multiple injections of each standard with various amounts .

The initial system for separation of the phospholipids and the gradient program is shown in Fig 1. (2) In all studies a 10 mm x 4.6 mm guard column was used.

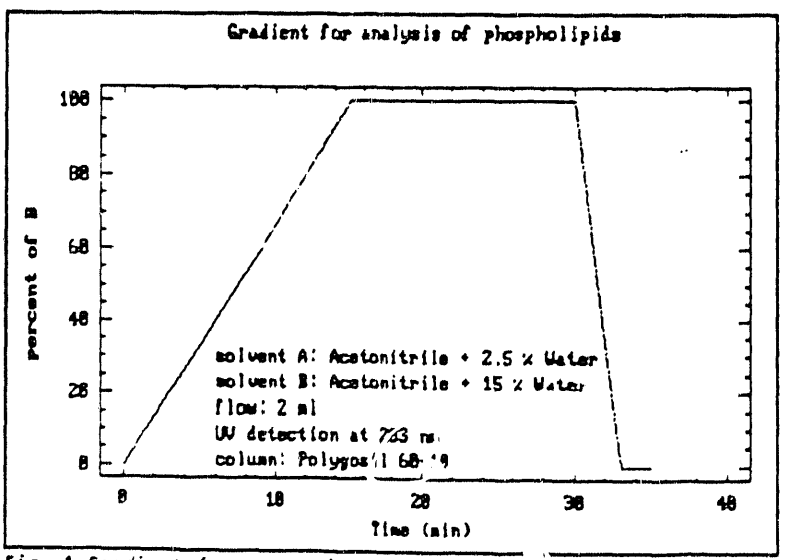

Fig. 1 Gradient for separation of phospholipids

Samples were collected with a fraction collector and then counted in an auto gamma counter with two window settings for the I-125 and I-131 photo peaks. The initial fractions (1-5) contained the neutral lipids which were pooled, evaporated and then analyzed on a second column after dissolution of the remaining compound in 25 µl chloroform/methanol 2:1.

The system and the gradient for separation of the neutral lipid fractions from the first column is shown in Fig 2.

RESULTS AND DISCUSSION

Thin-layer chromatography (TLC) is a convenient tool for analysis of the relative distribution of radioactivity in the various lipid pools after

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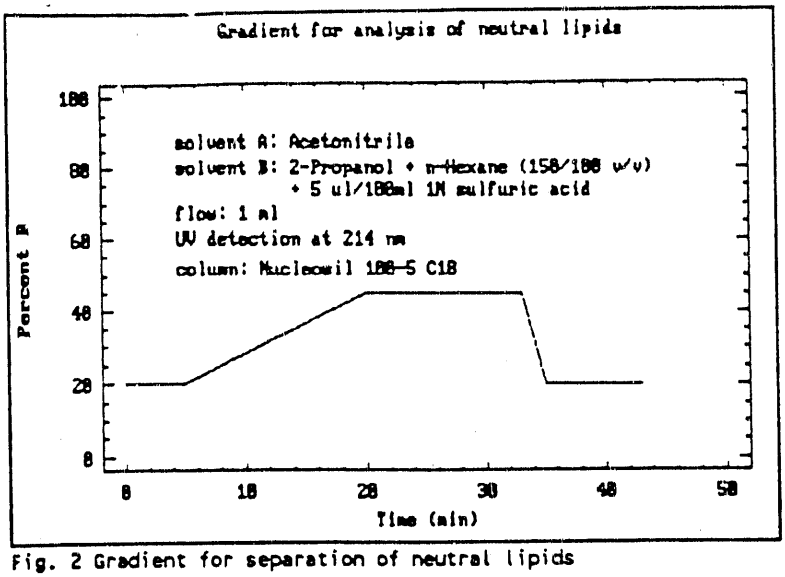

Fig. 2 Gradient for separation of neutral lipids

administration of radioiodinated analogues such as IPPA, BMIPP, and DMIPP to Langendorff-perfused hearts.

The resolution of the FFA, TG and PL classes is usually quite good by TLC, while the MG and DG migrate in the vicinity of other metabolites. Although the usual TLC systems allow a qualitative separation of all of the principal lipid classes on a single plate, special techniques are required to resolve the different PL classes. An understanding of the relative incorporation into different PL classes, however, is important.

Although total relative incorporation into the PL is usually lower (10-20%) for IPPA, BMIPP, and DMIPP than that in the TG pool (50-70%), a more detailed analysis of the relative incorporation into the principal phospholipid classes is important to evaluate the relative flux of these fatty acid analogues through the phospholipid biosynthetic machinery.

The SiO_2 column allowed separation of the principal phospholipids and the radioactive peaks which were observed (Figure 3) corresponded well to the mass detection of endogenous phospholipids. The early neutral lipid peak from these studies was concentrated and then further analyzed using a C-18 reversed phase column. Using this system the neutral lipid mixture was separated into the various lipid fractions. Due to the high complexity of the endogenous neutral lipids only regions of the retention times of the major classes match with the radioactivity in the fractions. For example, the difference in retention time of di-pentadecanoin and di-palmitin, which are different in only one CH_2 fragment in the fatty acid, was about 3 min. On the other hand, the time resolution of the 1 min. fractions was too low to match with peaks which had a FWHM of about 0.3 min. The results from these two systems are summarized in Figure 4 and 5 expressed as the relative percent of the total radioactivity. In the phospholipids there is only one major difference in uptake of the various tracers. IPPA is mostly incorporated into the lecithin whereas the major phospholipid for the uptake of the methyl-branched analogues BMIPP and DMIPP is cephalin. As expected, the greatest amount of radioactivity in the neutral lipids was found in the triglycerides. Surprisingly the uptake of DMIPP was balanced between triglycerides and monoglycerides which represent an intermediary metabolite during the formation of the TG storage lipids. In contrast to the other investigated fatty acids BMIPP was found in the FFA region of the chromatograms in a significant amount.

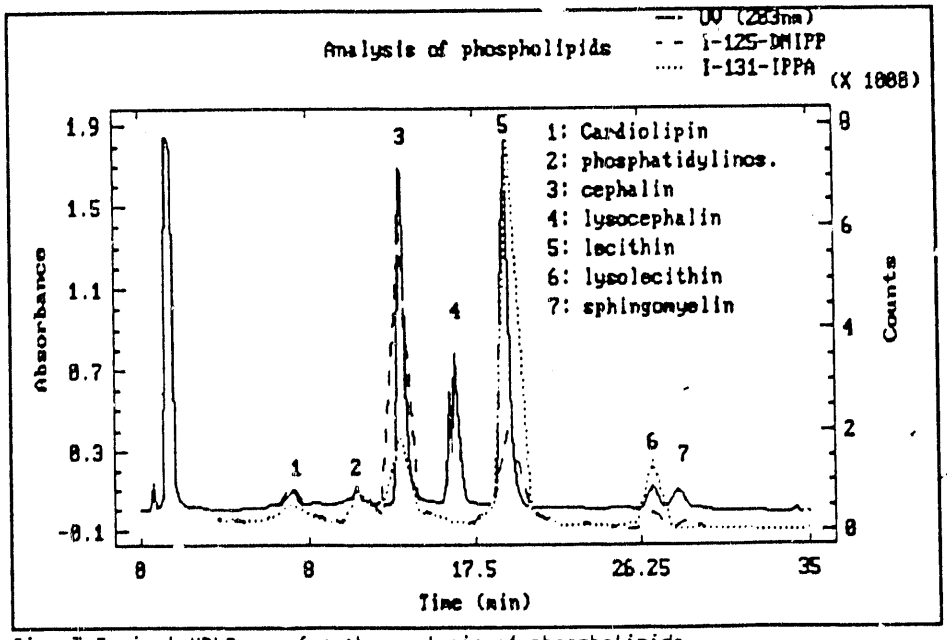

Fig. 3 Typical HPLC-run for the analysis of phospholipids

After calibration of both HPLC methods with standards, total amounts of the various lipid fractions could be calcualted quantitatively. Calibration was done by multiple injections of each standard with various amounts. The measured peak area was then plotted against the amount. From the slope and intercept each unknown amount could be computed. Figure 6 summarizes these data. The data of five hearts, normalized to the heart weight, are averaged. These data are in agreement with measurements of other groups, except for the diglycerides and the cholesterol for which Christie (1)

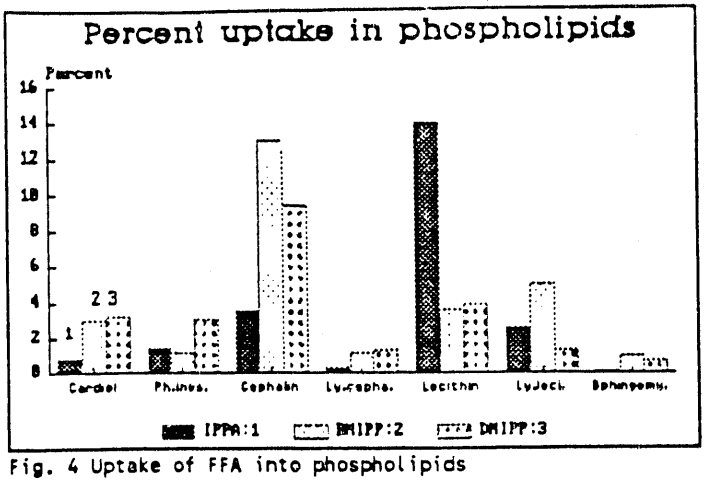

Fig. 4 Uptake of FFA into phospholipids

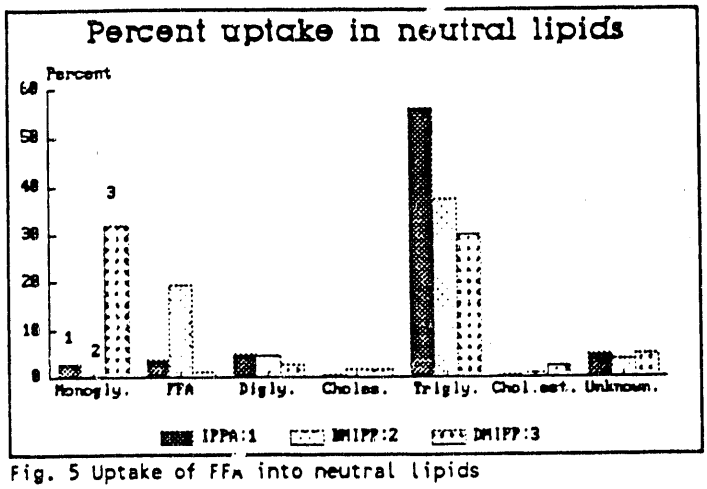

Fig. 5 Uptake of FFA into neutral lipids

found a ratio which is just opposite to our findings. The reasons might be that 1.): the retention times of these both neutral lipids are very similar so that a resolution in the chromatograms was very difficult and 2.): that the diglycerides had at least 4 components in the UV signal so that the differentiation to the cholesterol was limited.

Conclusion

HPLC methods are able to seperate and quantify as well phospholipids as neutral lipids. Most of the data are in agreement with data from the literature although the quantification of the neutral lipids revealed some limitations. The demonstration of incorporation of these fatty acids into the expected endogenous lipid pools would represent additional evidence that these modified fatty acids are extracted by myocytes and handled qualitatively like the endogenous FFA. These fatty acid analogues may therefore serve for imaging and quantification of myocardial metabolism in-vivo.

ACKNOWLEDGMENTS

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TOTAL AMOUNTS

Phospholipids (microgram)		Neutral lipids (microgram)	
Cardiolipin:	2744 +/-830	Monoglycerides:	304 +/-42
Phosfidylinos.:	8012 +/-731	Free fatty acids:	339 +/-9
Cephalin:	24424 +/-919	Diglycerides:	3208 +/-5
Lysocephalin:	4996 +/-412	Cholesterol:	440 +/-
Lecithin:	19208 +/-600	Triglycerides:	2800 +/-6
Lysolecithin:	4088 +/-864	Cholesterolester:	144 +/-3
Sphingomyelin:	1592 +/-462		
total:	65064 +/-4618	total:	7235 +/-1412
6.4% of total heart weight		0.8% of total heart weight	

Fig. 6 Amounts of endogenous lipids measured by calibrated peak areas

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