β-Methyl-15-p-iodophenylpentadecanoic acid metabolism and kinetics in the isolated rat heart

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Abstract. The use of 15-p-iodophenyl-β-methyl-pentadecanoic acid (βMe-IPPA) as an indicator of long chain fatty acid (LCFA) utilization in nuclear medicine studies was evaluated in the isolated, perfused, working rat heart. Time courses of radioactivity (residue curves) were obtained following bolus injections of both βMe-IPPA and its straight chain counterpart 15-p-iodophenyl-pentadecanoic acid (IPPA). IPPA kinetics clearly indicated flow independent impairment of fatty acid oxidation caused by the carnitine palmitoyltransferase I inhibitor 2-[5(4-chlorophenyl)pentyl]oxirane-2-carboxylate (POCA). In contrast, βMe-IPPA kinetics were insensitive to changes in fatty acid oxidation rate and net utilization of long chain fatty acid. Analysis of radiolabeled species in coronary effluent and heart homogenates showed the methylated fatty acid to be readily incorporated into complex lipids but a poor substrate for oxidation. POCA did not significantly alter metabolism of the tracer, suggesting that the tracer is poorly metabolized beyond βMe-IPPA-CoA in the oxidative pathway.

Key words: Radiiodinated fatty acid – β-oxidation – Myocardium – Perfusion

Methods

Stable IPPA and βMe-IPPA were obtained commercially (MARA Inc., Marcus Hook, PA), and radiolabeled in the melt, at 160°C and 130°C, respectively. 125I-Nal was transferred in ethanol from its commercial vial to a small, sealed, round bottom vial and dried under N2 gas at 30°C. Nonradioactive precursor was dissolved in ethanol at 30°C, added to the reaction vial and vortexed. After drying again, the vial was transferred to an oil bath for 1 h. Radiochemical yields were typically ~60%. TLC gave Rf = 0.35 for both acids. Specific radioactivities were 2–10 Ci/m mole. Radiolabeled fatty acids were purified and formulated as previously described (DeGrado et al. 1988).

Isolation and perfusion of rat hearts and external detection of 125I radioactivity were as previously described (DeGrado et al. 1988). Hearts were stabilized for a period of 25–30 min before rapid bolus injections (~25 µl) of radiiodinated fatty acids through a miniature catheter placed at the aortic root. The perfusion medium contained 5 mM glucose and 0.15 mM palmitic acid bound to 1% (equimolar) bovine serum albumin (Fraction V, Sigma Chemical, St. Louis, MO). Analysis of radioactivity in samples of coronary effluent and tissue homogenates were performed as previously described (DeGrado et al. 1988). Unidirectional extraction was estimated by treatment of the early residue data (0–2 min post injection) with conventional compartmental analysis using vascular and tissue compartments (Huang and Phelps 1986).

Results

In earlier studies with isolated, perfused, working rat hearts, we found that the externally detected tissue kinetics of 15-p-iodophenylpentadecanoic acid (IPPA) were sensitive to flow independent impairment of LCFA oxidation by the carnitine palmitoyltransferase I (CPT-I; EC 2.3.1.7) inhibitor, 2-[5(4-chlorophenyl)pentyl]oxirane-2-carboxylate (POCA) (DeGrado et al. 1988). Although changes in unidirectional extraction of IPPA were not evident in POCA treated hearts, early clearance patterns showed diminished washout of labeled products of β-oxidation. In the presently reported work, βMe-IPPA metabolism and kinetics were examined in this system in order to evaluate the usefulness of this compound as an indicator of LCFA oxidation in nuclear medicine studies with 123I.

IPPA residue curves from POCA hearts showed similar unidirectional extraction to controls (control − 0.251 ± 0.092,
POCA - 0.209±0.075) but more prolonged retention (Fig. 1A). Myocardial extraction of βMe-\(^{125}\)IPPA was slightly lower than for IPPA and not influenced by POCA (control - 0.171±0.074, POCA - 0.163±0.069). Radioactivity remaining in the supernatant of acid precipitated samples of coronary effluent reflected the clearance of labeled catabolite(s) from hearts (Fig. 2). In control hearts, early tissue clearance rates of radioactivity from βMe-\(^{125}\)IPPA (Fig. 1B) and clearance of catabolite(s) (Fig. 2) was slower than those of IPPA (DeGrado et al. 1988), demonstrating limited catabolism of the branched chain fatty acid. Although small decreases in the rate of production and clearance of catabolite(s) in POCA hearts were detected (Fig. 2), tissue residue curves were not altered (Fig. 1 C). Radioactivity distributions in control and POCA hearts at 1 min after injection (Figs. 3 and 4) were very similar, with low quantities of hydrophilic metabolites. Radioactivity in the organic fraction was identified as non esterified fatty acid (NEFA), triglyceride (TG), and phospholipid (PL). About 20% of the radioactivity was in the pellet fraction of homogenates of both control and POCA hearts. This fraction was not radiochemically identified.

**Discussion**

Neither βMe-IPPA residue curves nor radioactivity distributions in homogenates of hearts were significantly affected by POCA, although there was a slight slowing of the clearance of labeled catabolite(s). Since POCA inhibits fatty acid utilization at CPT-I, these data indicate that βMe-IPPA is poorly metabolized beyond βMe-IPPA-CoA in the oxidative pathway. They do not support the idea that βMe-IPPA-CoA is metabolized within the mitochondria to yield a trapped species which accumulates in proportion to the rate of β-oxidation of LCFA (Goodman et al. 1984; Knapp et al. 1986). These results corroborate those of Ambrose et al., who showed that the uptake and clearance of a β-methyl iodovinyl LCFA was governed primarily by uptake