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EFFECTS OF 2[5(4-CHLOROPHENYL)PENTYL]OXIRANE-2-CARBOXYLATE ON FATTY ACID AND GLUCOSE METABOLISM IN PERFUSED RAT HEARTS DETERMINED USING IODINE-125 16-IODOHEXADECANOATE

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Summary: Long-chain fatty acid oxidation by the isolated perfused rat heart was assayed by external counting using [^{125}I]16-iodohexadecanoic acid as substrate after administration of the hypoketonemic and hypoglycemic compound 2[5(4-chlorophenyl)pentyl]oxirane-2-carboxylate to rats. Glucose metabolism was also assessed by measuring release of tritium from [2-T]glucose. The oxidation of long chain fatty acids was virtually suppressed in hearts from fed or starved rats given 2[5(4-chlorophenyl)pentyl]oxirane-2-carboxylate while glucose utilization was increased 2-2.5 fold.

Introduction: Fatty acids are a major fuel for the mammalian heart, and maximum cardiac work depends on fatty acid oxidation (1,2). POCA has powerful hypoketonemic and hypoglycemic activities (3,4), and is a candidate hypoglycemic and cardioprotective drug. The CoA ester of POCA (POCA-CoA) is a powerful inhibitor (I-50 less than 1 micromolar) of the oxidation of palmitoyl-CoA, but not of palmitoyl-carnitine, by rat liver and skeletal mitochondrial fractions thus localising the inhibition of beta-oxidation at the stage of carnitine palmitoyltransferase I (EC 2.3.1.21) situated on the outer face of the inner mitochondrial membrane (5,6,7). Rosen and Reinauer (8) showed that 1 mM POCA added to the perfusion fluid in a perfused rat heart preparation inhibited the formation of $^{14}CO_2$ from [1-^{14}C]palmitate and also increased the proportion of pyruvate dehydrogenase (EC 1.2.4.1) in the active

Abbreviations: POCA, 2[5(4-chlorophenyl)pentyl]oxirane-2-carboxylate; IHDA, [^{125}I]16-iodohexadecanoate.

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form. Chronic administration of POCA to rats (0.05-0.2%, w/w in diet) for 12 weeks caused a mild cardiac hypertrophy of about 15-20% of the wet weight of the heart, without causing any apparent histochemical or ultrastructural changes (9). It was of interest, therefore, to determine directly the effects of administration of POCA to rats on the metabolism of long-chain fatty acids and of glucose in hearts. For this purpose IHDA is a useful indicator compound using external detection of total heart radioactivity (10).

Methods: POCA (Na salt) was generously provided by Dr Gerhard Ludwig, Byk GuldenLomberg Chemische Fabrik GmbH, Konstanz, FRG. IHDA was prepared before use by isotope exchange (10), at a specific radioactivity of 1 Ci/mmol. Female Sprague-Dawley rats weighing about 230g were used. POCA was administered intraperitoneally in a dose of 30 mg per kg body-wt as a solution of its sodium salt, either to fed rats or rats which had been fasted for 18 hr. Ten minutes before use the rats received 750 units of heparin intraperitoneally. They were anesthetised with ether and the hearts perfused by the Langendorff technique with Krebs-Ringer phosphate solution containing 0.6mM glucose, with the force and frequency of contraction recorded with a pressure transducer. Heart contractions were apparently normal after POCA treatment, although the flow rate was slightly increased. After about 5 minutes 0.3 µCi per ml of [2-T]glucose was added to the perfusion medium; samples of the outflow were collected 10, 12, and 14 minutes later. The perfusate was passed through columns (1 x 5cm) of Dowex 1 (200-400 mesh) in the borate form to remove unchanged glucose and the tritiated water released counted by liquid scintillation as an abolute measure of exogenous glucose metabolised by glycolysis and oxidation. Then IHDA (50µCi dissolved in 20µl of Tween 80/ethanol/0.9% NaCl, 1:4:35) was injected close to the coronary arteries and the radioactivity monitored by scintillation probes positioned 6.5cm apart with the heart between them. Count rates from the probes were summed and recorded with a minicomputer.

Results: The time courses of the duration of radioactivity in hearts from a fed control and a fed POCA-treated rat following a bolus of IHDA are shown in Fig. 1. Mean extractions ranged from 80 to 90% of the dose of IHDA for all

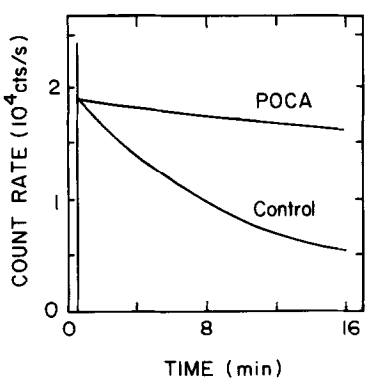

Figure 1: Time course of ^{125}I in hearts after bolus administration of IHDA.

Table 1: Effects of POCA on glucose uptake and fatty acid turnover.

Group	Fatty acid turnover				Glucose uptake
	Extrn	A1/(A1+A2)	k1 sec^{-1}	k2 sec^{-1}	μmol/min
Fasted controls	82(7)	0.81(0.06)	1.96(0.14) $x10^{-3}$	3.34(1.44) $x10^{-4}$	0.19(0.14)
Fasted POCA	83(8)			1.47(0.40) $x10^{-4}$	0.40(0.03)
Fed controls	80(10)	0.84(0.07)	1.84(0.11) $x10^{-3}$	1.79(0.96) $x10^{-4}$	0.19(0.02)
Fed POCA	89(1)			7.91(0.91) $x10^{-5}$	0.52(0.09)

Values are the mean (standard deviation) for 3 hearts for POCA treated and fed control groups, for 24 hearts for fasted control glucose uptake data and for 5 hearts for fasted control IHDA data.
A1/(A1+A2) is fraction of extracted label which clears with rate-constant k1.
k1 and k2 are rate-constants for the observed fast and slow phases of clearance, respectively.

conditions (Table 1). With hearts from control animals there was the expected rapid efflux of radio-activity indicative of the metabolic conversion of the iodine of IHDA to iodide which is washed out of the tissue (11). By contrast, the rate of efflux of radioactivity was dramatically less in the hearts from POCA-treated animals indicating virtually complete inhibition of long-chain fatty acid oxidation. The clearance phases of radioactivity-time curves for fed and fasted control hearts were very similar and were well described by two exponential processes whose rate-constants k1 and k2 are given in Table 1. The fraction of label which cleared according to the more rapid process (k1) is given as A1/(A1+A2) and was close to 80%. The curves for hearts from POCA-treated rats could be described as single exponentials whose rate-constants were a little smaller than the slower of the two components (k2) found in control hearts (Table 1). The loss of tritium from [2-T]glucose was greater with the hearts from POCA-treated rats than in the controls (Table 1).

Discussion: This work provides a direct demonstration that pretreatment of rats with doses of POCA which promote hypoketonemia and hypoglycemia in the

fasted state (3,4) causes virtually complete inhibition of long-chain fatty acid oxidation in the heart. It is not yet known whether the inhibition of carnitine palmitoyltransferase by POCA-CoA is reversible (8), but the activity of the enzyme is low in skeletal muscle mitochondrial fractions prepared from POCA-treated rats. These data do not differentiate between IHDA retained in the hearts as the free acid, its CoA-ester or esterified in complex lipids.

The results are expressed in terms of rate-constants, rather than in abolute amounts of fatty acid metabolised, since the size of the pool of free fatty acids is not known. However, it is assumed that this is similar in the treated and untreated animals after administration of heparin. Furthermore, the efflux of radio-iodine from the heart is probably rate-limited by diffusion of iodide from the mitochondrial matrix (12), rather than by beta oxidation. Rate-constants (k1) for control hearts given in Table 1 should therefore be regarded as lower limits, and the inhibition of beta oxidation by POCA may be greater than apparent from Fig. 1.

The increase in glucose utilisation by hearts from POCA-treated rats is consistent with an increased dependence on carbohydrate metabolism secondary to impaired fatty acid oxidation. It was not possible to determine the relative contributions of glycogen and of exogenous glucose. Further, the extent of mitochondrial oxidation of pyruvate formed by glycolysis is not known. However, the activation of pyruvate dehydrogenase by POCA reported previously (6), arising presumably from decreased generation of NADH and acetyl-CoA due to impaired fatty acid oxidation, would facilitate pyruvate oxidation. The increments of glucose oxidation found were relatively small but this may be expected since the heart preparation used only did a small amount of mechanical work.

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