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PRELIMINARY COMMUNICATION

SYNTHESIS OF 3-PHENOXYBENZOIC ACID-CONTAINING LIPIDS VIA THE MONOACYLGLYCEROL PATHWAY

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The incorporation of xenobiotic acids into mammalian lipids has been reported, both *in vivo* (1) and in liver slice experiments (2). 3-Phenoxybenzoic acid (3PBA), a metabolite of many pyrethroid insecticides, including permethrin and decamethrin, is one such lipophilic xenobiotic. Using a rat liver microsome preparation we have been able to demonstrate that 3PBA is incorporated into neutral lipids by enzymes of the monoacylglycerol pathway.

MATERIALS AND METHODS: [^{14}C]3PBA, labelled in the benzoyl ring, was synthesised at the Sittingbourne Research Centre and from this [^{14}C]3PBA-CoA was prepared by a mixed anhydride method (3). [9,10-^{3}H]Palmitic acid was purchased from Amersham International plc. 2-Monopalmitoylglycerol (2-MPG) was obtained from P.L. Biochemicals Ltd., 1-monopalmitoylglycerol (1-MPG) from Sigma Chemical Company and 2-hexadecylglycerol (2-HDG) was a gift from Dr. D.N. Brindley. Emulsions of these compounds were prepared in 0.6 mg/ml fatty acid free bovine serum albumin using an ultrasonic disintegrator.

Standard assays for acyl-CoA:monoacylglycerol *O*-acyltransferase (EC2.3.1.22) contained, in a final volume of 0.25 ml, 25mM Hepes buffer pH 7.4, 6 mg/ml bovine serum albumin, 6mM $MgCl_2$, 2mM 2-HDG and rat liver microsomes (1.2 mg protein/ml) with either (a) 250μM 3PBA-CoA or (b) an acyl-CoA generating system consisting of 0.6mM 3PBA, 2.4mM ATP, and 55μM CoA. Parallel incubations contained 0.6mM potassium [9,10-^{3}H]palmitate in place of 3PBA in system (b) above. Dithiothreitol appeared to promote rapid hydrolysis of 3PBA-CoA so this was not included in the assays and thiol groups were protected from oxidation by purging all solutions with N_2. The reactions were started by the addition of liver microsomes and incubated at 37°C for 30 min. before they were stopped by the addition of 1.88 ml chloroform/methanol (1:2 v/v).

Lipids were extracted by the method of Bligh and Dyer (4) and the labelled acidic precursors were separated from the neutral lipid products by column chromatography on basic alumina, the latter being eluted in chloroform. The radioactive content of the neutral lipid fraction was determined by liquid scintillation counting. Tlc was carried out using Merck silica gel 60F plates developed in hexane:diethyl ether: acetic acid (70:30:1 by vol.). Appropriate standards were applied to the plates and detected by exposure to iodine vapour. Labelled compounds were quantified as described by Sanchez *et al* (5).

Field desorption mass spectroscopy was performed on a Varian 731 mass spectrometer.

RESULTS: The 2-HDG dependent incorporation of [^{14}C]3PBA into neutral lipids by rat liver microsomes required the presence of a 3PBA-CoA intermediate: incubations containing 3PBA produced ^{14}C labelled neutral lipids only when CoA and ATP were present. A control, in which boiled microsomes replaced the usual enzyme source, also resulted in no incorporation into neutral lipids. The rate of incorporation using acyl-CoA generated *in situ* was approximately 1/15th that observed when exogenous 3PBA-CoA was used, therefore 3PBA-CoA was added routinely to the assays.

The incorporation of 3PBA into neutral lipids was dependent upon protein concentration and time of incubation, the rate being linear up to 1.2 mg protein/ml of assay and 40 min. Incorporation of label also varied with the concentration of both acyl donor and acyl acceptor; optimum activity was obtained with 250μM 3PBA-CoA (fig 1) and 2mM 2-HDG (fig 2). Although [^{3}H]palmitate was also incorporated into neutral lipids under the above conditions, this activity was not dependent on 2-HDG. Substituting 2-MPG as the acyl acceptor also gave concentration dependent incorporation of 3PBA into neutral lipids, this physiological substrate had a higher apparent Km than its alkylglycerol analogue (fig 2). When the 1-monopalmitoyl isomer was used very little activity was detected; at a concentration of 2mM monopalmitoylglycerol the rate for the 1-isomer was 1/17th that of the 2-isomer.

Analysis of the 2-HDG/3PBA containing lipids by TLC showed that 70% of the labelled compound co-chromatographed with a dioleoylglycerol standard and 30% with trioleoylglycerol. Treatment of the products with diazomethane, a reagent that methylates only free acids, gave the same chromatographic profile, indicating that there was no contamination of the product with free 3PBA. Alkaline hydrolysis followed by treatment with BCl_3 in methanol resulted in all the label co-chromatographing with methyl-3PBA as expected.

The field desorption mass spectrum showed that the 'diacylglycerol' component contained a compound with m/z consistent with 1-(3-phenoxybenzoyl)-2-hexadecylglycerol. The 'triacylglycerol' fraction had a component with the correct m/z for *rac*-1-(3-phenoxybenzoyl)-2-hexadecyl-3-palmitoylglycerol.

DISCUSSION: The incorporation of [^{14}C]3PBA into neutral lipids is dependent upon the formation of 3PBA-CoA as an intermediate. 2-HDG and 2-MPG were both active as substrates for the enzyme whereas the 1-acylglycerol had a much lower affinity. The mass spectral data indicated the presence of 1-(3-phenoxybenzoyl)-2-hexadecylglycerol as the product and this, taken with the substrate requirements described above, shows the reaction is catalysed by a genuine monoacylglycerol acyltransferase rather than being a reversal of lipolysis (6). The production of a triacylglycerol analogue suggests that diacylglycerol acyltransferase can also make use of 3PBA-containing substrate(s).

These experiments indicate that the formation of the 3PBA-CoA ester is the rate limiting step in the reaction sequence. Thus tissues and species with a higher acyl-CoA synthetase activity may have a greater potential for the synthesis of xenobiotic diacylglycerols and triacylglycerols.

This discovery of the incorporation of 3PBA into lipids via monoacylglycerol acyltransferase is surprising as the enzyme is not normally active in the liver of adult rats (7). This, to our knowledge, is the first demonstration of an enzymatic step through which xenobiotic acids can be incorporated into glycerolipids.

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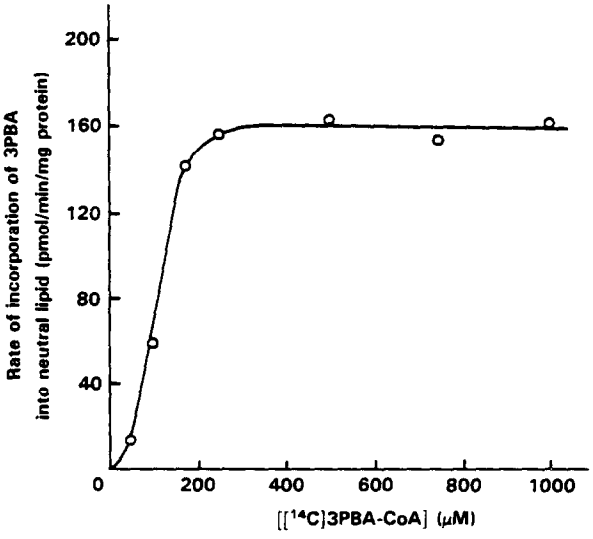

Fig 1 Effect of 3PBA-CoA concentration on the incorporation of 3PBA into neutral lipids

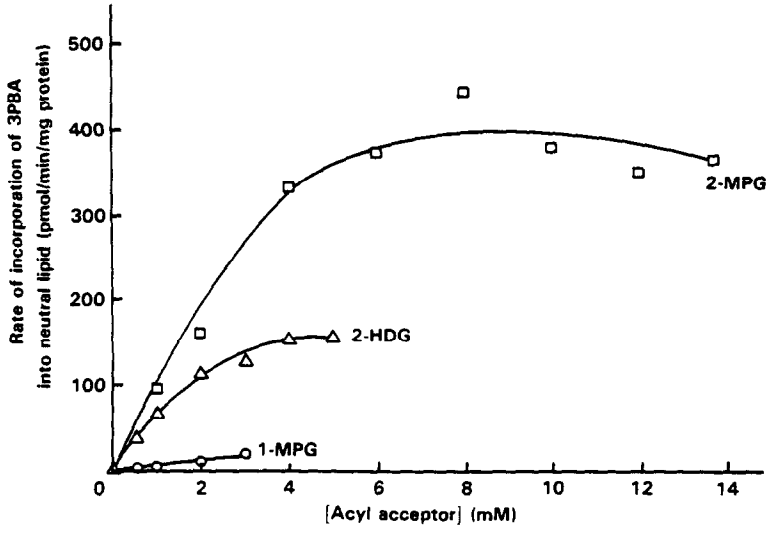

Fig 2 Rates of incorporation of 3PBA into neutral lipid by monoacylglycerol acyltransferase using 2-HDG, 2-MPG and 1-MPG as substrates