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SHORT COMMUNICATIONS

Effects of 2[5(4-chlorophenyl)pentyl]oxirane-2-carboxylate on lipoprotein lipase, adipose tissue lipolysis and glycerol phosphate acyltransferase in rats

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2[5(4-Chlorophenyl)pentyl]oxirane-2-carboxylate (POCA) is a candidate anti-diabetic drug which has strong hypoketonaemic and hypoglycaemic effects [1]. One effect of the drug *in vivo* is to lower the plasma triacylglycerol concentration [2] and part of the present work was to investigate whether POCA acts on the removal of triacylglycerol from the blood through changes in LPL (EC 3.1.1.34) activity. A second aspect was to study the effects of POCA on adipose tissue lipolysis to see whether the provision of fatty acid for triacylglycerol synthesis was altered.

A possible common feature of POCA action is through its conversion to POCA-CoA and interference in enzyme reactions involving CoA derivatives as substrates or regulators [3]. Glycerophosphate acyltransferase (acyl CoA: L-glycerol 3-phosphate-*O*-acyltransferase, EC 2.3.1.15) is such an enzyme in triacylglycerol and phospholipid metabolism and the effect of POCA-CoA on this enzyme was also studied.

Materials and methods

Collagenase (Type 11), bovine serum albumin (Fraction V), were obtained from Sigma Chemical Co. and L-[U-^{14}C] glycerol 3-phosphate from Amersham International. POCA (sodium salt) was a gift from Dr Gerhard Ludwig, Byk Gulden Lomberg Chemische Fabrik GmbH (Konstanz, F.R.G.). POCA-CoA was kindly provided by Dr H. S. A. Sherratt, Newcastle upon Tyne University [3].

Male Porton Wistar rats (200–250 g) were fed either standard laboratory chow pellets *ad libitum* or, for 3 nights, chow pellets impregnated with POCA (0.2%, w/w). Pellets were prepared daily by spraying with either 10% POCA in acetone or acetone alone [2].

Fresh hearts and acetone–ether preparations of epididymal adipose-tissue [4] (tissue from 2 rats per powder) were homogenised in 0.25 M NH_3/NH_4 Cl buffer pH 8.1 and assayed for LPL [5]. For measurement of serum PHLA, rats were injected in the tail vein with heparin (10 I.U./kg) and bled from the dorsal aorta after 5 min while under light ether anaesthesia.

Fat cells were prepared by the method of Rodbell [6]. Epididymal fat pads or adipocytes (3.7×10^5 cells/ml) were incubated in Krebs–Ringer bicarbonate buffer containing 5% defatted albumin [7] and 5 mM glucose [8]. Fat cells were separated from the medium by centrifugation (220 *g* for 5 min) through 1 ml of silicone oil, (200/50 cs Dow Corning). Adipose tissue, medium and cell extracts for glycerol assay were prepared in 3% perchloric acid followed by neutralisation with 3.5 mM NaOH [8]. Adipocyte cell number is expressed in relation to lactate dehydrogenase activity where 1 unit of enzyme is equivalent to 1×10^5 cells.

Glycerol phosphate acyltransferase activity was measured in the microsomal fraction of liver [9]. The final concentration of glycerophosphate was 3 mM. Protein was measured by the method of Lowry *et al.* [10], glycerol by the method of Garland and Randle [11], serum triacylglycerol by the method of Fletcher [12], free fatty acid by the Dole extraction procedure [13]. Results are means ± SD. Significance of difference from controls is determined by Student's *t*-test except where stated.

Results and discussion

Three nights of drug treatment were chosen as there was a significant decrease in the plasma triacylglycerol concentration from 0.87 ± 0.33 mM in control animals to 0.27 ± 0.05 mM ($N = 12$, $P < 0.01$) by this time.

The results in Table 1 show that POCA treatment caused a significant increase in plasma LPL activity. This finding suggests that the fall in plasma triacylglycerol concentration after POCA-treatment could, in part, be explained by an increase in the body's capacity for triacylglycerol removal. The observed significant increase in heart enzyme activity with no change in adipose tissue enzyme activity further supports this conclusion (Table 1).

The effect of POCA *in vivo* on the lipolytic rate was measured *in vitro* from the rate of glycerol release from epididymal fat pads of control and drug-treated rats. There was a three fold increase in the net glycerol production from tissue from POCA-treated as compared with that from control animals (Table 2). When adipocytes from control animals were incubated for 4 hr in the presence of 0.05 mM POCA the net glycerol change in medium and cells was significantly higher, on a paired basis, than the change in its absence (Table 2). The effect of POCA to increase lipolysis is a surprising action for a drug which results in the lowering of the plasma triacylglycerol concentration. However, there was no consistent increase in FFA release from adipose tissue (Table 2) and the extra FFA produced may be re-esterified. As POCA was found to interfere with the measurement of free fatty acid by the titration method, meaningful results for free fatty acid release from adipocytes could not be obtained. POCA did

Table 1. Effects of POCA on LPL activity

	Control	POCA-treated
Post-heparin plasma LPL (μmol free fatty acid/hr/ml)	9 ± 2	13 ± 3*
Heart LPL (μmol free fatty acid/hr/g)	39 ± 12	56 ± 7*
Adipose tissue LPL (μmol free fatty acid/hr/g)	38 ± 14	44 ± 15

Post-heparin plasma LPL activity is calculated as the difference in total serum lipolytic activity and the lipolytic activity in the presence of sodium chloride (1 M final concentration). Values are given as means ± SD for 12 animals. *$P < 0.01$ by Student's *t*-test.

* Abbreviations used: FFA, free fatty acid; LPL, lipoprotein lipase; POCA, 2[5(4-chlorophenyl)pentyl]oxirane-2-carboxylate; POCA-CoA, 2[5(4-chlorophenyl)-pentyl]-oxirane-2-carbonyl-CoA.

Table 2. Net changes in glycerol and FFA

	Glycerol (μmol/hr/g)	FFA (μmol/hr/g)
Adipose tissue		
Control	0.31 ± 0.2	0.21 ± 0.3
POCA-treated	0.99 ± 0.43*	0.51 ± 0.63
	nmol/hr/10^5 cells	
Adipocytes		
No addition	8.0 ± 5.3△	
POCA 0.05 mM	13.0 ± 5.3	

Measurements were made in the medium, adipose tissue and adipocytes at the beginning and end (1 hr for fat pads, 4 hr for cells) of the incubations. The glycerol and FFA production calculated as net change in medium + tissue or medium + cells. Values are given as means ± SD for 6 animals for adipose tissue and for 4 different cell batches. * $P < 0.01$; △ significant by paired *t*-test.

not increase the plasma free fatty acid concentration which was 0.54 ± 0.24 mM in the drug-treated animals compared to 0.49 ± 0.14 mM in controls (N = 12). This indicates that either there is no increase in the rate of release of FFA from adipose tissue *in vivo* or that the rate of FFA utilization is increased proportionately. Increased lipolysis would fit with the finding of lower total body fat content of rats fed 0.2% POCA in their diet for 12 weeks [2].

After POCA treatment there was no significant change in the activity of glycerol phosphate acyltransferase in the microsomal fraction of liver (control, 3.9 ± 2.6; POCA-treated, 7.9 ± 2.3; nmol product/min/mg protein, N = 8). The addition of POCA-CoA to the assay of microsomal glycerol phosphate acyltransferase did not alter the enzyme's activity (control, 7.2 ± 2.8; with 0.05 mM POCA-CoA, 6.5 ± 3.1; with 0.1 mM POCA-CoA, 6.1 ± 2.0; nmol product/min/mg protein, N = 4).

The work presented here shows that, compared with controls, feeding 0.2% POCA for 3 nights increased LPL activity in heart and post heparin plasma with no change in adipose tissue enzyme activity, suggesting increased triacylglycerol removal in drug-treated animals. POCA-feeding and incubation of isolated adipocytes with 0.05 mM POCA increased triacylglycerol lipolysis as evidenced by increased glycerol production. Glycerol phosphate acyltransferase activity was not inhibited on incubation with 0.05 mM POCA-CoA showing that the drug does not inhibit all acyl CoA reactions.

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REFERENCES

1. H. P. O. Wolf, K. Eistetter and G. Ludwig, *Diabetologia* **22**, 456 (1982).
2. P. P. Koundakjian, D. M. Turnbull, A. J. Bone, M. P. Rogers, S. I. M. Younan and H. S. A. Sherratt, *Biochem. Pharmac.* **33**, 465 (1984).
3. K. Bartlett, D. M. Turnbull and H. S. A. Sherratt, *Biochem. Soc. Trans.* **12**, 688 (1984).
4. P. Ashby, A. M. Tolson and D. S. Robinson, *Biochem. J.* **171**, 305 (1978).
5. S. E. Riley and D. S. Robinson, *Biochim. biophys. Acta* **369**, 371 (1974).
6. M. Rodbell, *J. biol. Chem.* **239**, 375 (1964).
7. R. F. Chen, *J. biol. Chem.* **242**, 173 (1967).
8. M. Vaughan, *J. biol. Chem.* **237**, 3354 (1962).
9. E. J. Bates and D. S. Saggerson, *FEBS Lett.* **84**, 229 (1977).
10. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
11. P. Garland and P. J. Randle, *Nature, Lond.* **196**, 987 (1962).
12. M. J. Fletcher, *Clin. chim. Acta* **22**, 393 (1968).
13. V. P. Dole and H. Meinertz, *J. biol. Chem.* **235**, 2595 (1960).

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Organosulfur oxygenation and suicide inactivation of lactoperoxidase*

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Our recent report demonstrated the involvement of peroxidases in the oxidative metabolism of organosulfur compounds [1]. Lactoperoxidase (LPX) catalyzed the oxygenation of organosulfide and thioamide functional groups to the respective sulfoxide. The enzymatic reactivity correlated with the peak potential for electrochemical oxidation of the substrates.

These observations provided a clue to the mechanism of thiocarbamide goitrogen-induced inactivation of LPX and the closely related thyroid peroxidase [2]. The results of our subsequent study supported a suicide inactivation mechanism for the action of thiocarbamides on LPX [3].

This communication presents data that extend the range of observations on the S-oxygenation reactions catalyzed by LPX and give additional support to a causal relationship between thiocarbamide oxygenation and suicide inactivation.

Methods

2-Mercaptobenzothiazole (I), obtained from the Pfaltz & Bauer Co., was recrystallized from methanol. 6-Propyl-2-thiouracil (II), obtained from the Sigma Chemical Co., was recrystallized from aqueous ethanol. Phenyldisulfide [4], 2,2′-bis-dithiobenzothiazole (MBTS) [4], phenacyl-phenyl sulfide (PPS) [5], and the corresponding sulfoxide (PPSO) [6] were synthesized according to published procedures. Bovine LPX was obtained from the Sigma Chemi-

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