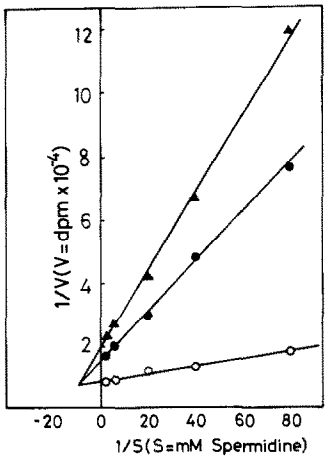

Fig. 3. Inhibition of spermine synthase by 5′-*S*-isobutyl-5′-deoxyadenosine with spermidine as the variable substrate. Spermine synthase activity was assayed in the absence (○) or presence of 25 μM (●) or 50 μM (▲) 5′-*S*-isobutyl-5′-deoxyadenosine, 40 μM decarboxylated *S*-adenosylmethionine and 0.0125–0.5 mM spermidine and 97 μg enzyme protein.

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Biochemical Pharmacology, Vol. 31, No. 8, pp. 1650–1652, 1982.
Printed in Great Britain.

0006-2952/82/081650–03 $03.00/0

Effect of chronic clofibrate feeding on the activities of enzymes involved in glycerolipid synthesis and in peroxisomal metabolism in rat liver

(*Received* 14 *September* 1981; *accepted* 11 *November* 1981)

Dihydroxyacetone phosphate is an alternative precursor to glycerol phosphate for the biosynthesis of glycerolipids in mammalian cells [1–3]; although the physiological importance of these two routes of metabolism is not established. The initial esterification of fatty acids to phosphatidate could involve three different acyltransferases: (a) a glycerol phosphate acyltransferase (EC 2.3.1.15) located in the endoplasmic reticulum which can also use dihydroxyacetone phosphate as an acyl acceptor [4, 5]; (b) a specific glycerol phosphate acyltransferase located in the mitochondrial outer membrane [6–9]; and (c) a specific dihydroxyacetone phosphate acyltransferase that is found in peroxisomes [10–12].

The latter activity has only recently been described and its possible importance in producing diacylglycerolipids is unknown.

The present work was performed to investigate how the peroxisomal acyltransferase changes in activity in relation to other enzymes of phosphatidate metabolism when the peroxisomal content of the liver is modified. This was achieved by feeding rats with a metabolite of the hypolipidaemic drug, clofibrate, which is known to produce peroxisomal proliferation in the liver.

Sodium-4-chlorophenoxyisobutyrate was a gift from Imperial Chemical Industries Ltd. (Macclesfield, U.K.), the source of other materials, the rats, and the conditions of enzymic analysis in liver homogenates is described elsewhere [13]. For some incubations the liver homogenate was preincubated with *N*-ethylmaleimide [13] to inhibit the glycerol phosphate acyltransferase of the endoplasmic reticulum that can also esterify dihydroxyacetone phosphate [4, 5]. The *N*-ethylmaleimide-insensitive glycerol phosphate acyltransferase is mitochondrial [11, 14–16], whereas the insensitive dihydroxyacetone phosphate acyltransferase is mainly peroxisomal [10–12]. This latter activity was measured by using a concentration of 400 μM palmitoyl-CoA rather than 523 μM which was used previously [13].

The standard 41B diet [13] was crushed and a solution of sodium chlorophenoxyisobutyrate was mixed with the powder to form a stiff paste with a final concentrate of 5 g of drug per kg of dry diet. The mixture was reconstituted

Table 1. Effect of 4-chlorophenoxyisobutyrate treatment on some hepatic activities

Activity	nmoles of substrate converted/min/mg of liver DNA		
	Control	Drug treated	Significance
Total glycerol phosphate acyltransferase	201 ± 19	395 ± 56	$P < 0.001$
Mitochondrial glycerol phosphate acyltransferase	72 ± 23	140 ± 23	$P < 0.001$
Microsomal glycerol phosphate acyltransferase	133 ± 25	257 ± 63	$P < 0.001$
Peroxisomal dihydroxyacetone phosphate acyltransferase	11 ± 1	22 ± 3	$P < 0.001$
Soluble phosphatidate phosphohydrolase	61 ± 12	60 ± 15	NS
Acyl-CoA oxidase	406 ± 62	1781 ± 145	$P < 0.001$
Urate oxidase	1314 ± 197	1804 ± 342	$P < 0.005$

Results are given as means ± S.D. for 8 control and 7 treated rats.
NS, not significant.

into pellets which were dried for 2 days at 37°. Groups of three or four rats were housed in grid-bottomed cages, and they were fed with the experimental, or reconstituted diet (controls) for 10 days.

The rats fed with chlorophenoxyisobutyrate has an initial body weight of 188 ± 7 g (mean ± S.D.) and they gained 17 ± 5 g/100 g of body weight over the 10 days of treatment. This was less ($P < 0.05$) than the 23 ± 6 g/100 g gained by the control rats, although the food consumption was not significantly different. As expected, the drug increased ($P < 0.001$) the liver weights from 10.7 ± 1 g in the controls to 14.0 ± 0.6 g in treated rats. The liver protein contents were also increased (2.06 ± 0.46 g compared with 3.43 ± 0.43 g; $P < 0.001$). Hepatic DNA concentrations were not significantly altered and the enzyme activities in Table 1 are expressed relative to DNA.

The activity of acyl-CoA oxidase was increased by 4.4-fold after feeding the drug and this was anticipated from previous work [17, 18]. This enzyme catalyses the first reaction of peroxisomal β-oxidation, and its increase was much greater than was that of urate oxidase (EC 1.7.3.3) which is another peroxisomal maker (Table 1). There was a 2-fold increase in the activity of the *N*-ethylmaleimide-insensitive dihydroxyacetone phosphate acyltransferase, which was intermediate between the increases for acyl-CoA oxidase and urate oxidase. Increases of about 2-fold were also obtained for the total, microsomal and mitochondrial glycerol phosphate acyltransferase activities (Table 1). An increase in total glycerol phosphate acyltransferase activity has been previously reported [19], and a slight increase in glycerol phosphate acyltransferase activity was shown in hepatocytes obtained from rats treated with clofibrate [20].

The subsequent metabolism of the phosphatidate that is formed by the various acyltransferases is catalysed by phosphatidate phosphohydrolase (EC 3.1.3.4). This enzyme is thought to have a regulatory function in the liver, especially in facilitating an accelerated synthesis of triacylglycerols [21]. Treatment of the rats with chlorophenoxyisobutyrate failed to alter this activity (Table 1).

The importance of the results in Table 1 in terms of effecting the hypolipidaemic action of clofibrate is difficult to assess. Clofenapate, an analogue of clofibrate, can acutely inhibit the esterification of dihydroxyacetone phosphate [22]. If it were also to increase the activity of dihydroxyacetone phosphate acyltransferase in the long term, then this might serve to compensate for this inhibition. Clofibrate can also directly inhibit some acyltransferase activities [23, 24], but it is far less potent than clofenapate [25].

It has been suggested that the increase in activity of carnitine palmitoyltransferase which is produced by clofibrate treatment could promote fatty acid oxidation in motochondria, since this increase is greater than that for glycerol phosphate acyltransferase [19]. Similarily, the increase in acyl-CoA oxidase activity is twice that for dihydroxyacetone phosphate acyltransferase (Table 1), and this could favour the β-oxidation in peroxisomes. However, the latter process is thought to account for less than 10% of the total oxidation of palmitate and oleate [18]. It is also uncertain to what extent the peroxisomal system contributes to the esterification of fatty acids. Acyldihydroxyacetone phosphate is an obligatory intermediate for the synthesis of alkyl- and alkenyl- lipids. The peroxisomal acyltransferase could also be responsible for the relatively high utilization of dihydroxyacetone phosphate as a precursor for the diacylglycerolipids [1–3]. Peroxisomes are found in association with the endoplasmic reticulum [26, 27] where most of the glycerolipid synthesis is thought to occur. A further function of acyldihydroxyacetone phosphate could be to act as a carrier for activated fatty acids in the cell [28].

Feeding rats with chlorophenoxyisobutyrate increased the peroxisomal activities of acyl-CoA oxidase, urate oxidase, and dihydroxyacetone phosphate acyltransferase by 4.4, 1.4 and 2-fold respectively. The total, microsomal and mitochondrial glycerol phosphate acyltransferases were increased in activity by about 2-fold, whereas the phosphatidate phosphohydrolase activity did not change. The alterations in the relative activities of enzymes involved in fatty acid oxidation and esterification might contribute to the hypolipidaemic action of clofibrate.

Acknowledgements—We thank the Science Research Council for providing a studentship to A.D.P. and the Medical Research Council for providing a project grant.

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Biochemical Pharmacology, Vol. 31, No. 8, pp. 1652–1653, 1982.
Printed in Great Britain.

0006-2952/82/081652-02 $03.00/0

Effect of BW755C on prostaglandin synthesis in the rat stomach

(*Received* 18 *August* 1981; *accepted* 12 *November* 1981)

Recently Whittle *et al.* [1, 2] have shown that the dual inhibitor of cyclooxygenase and lipoxygenase, BW755C [3-amino-1-(3-trifluoromethylphenyl)-2-pyrazoline], inhibits prostaglandin (PG) production in inflammatory exudates of rats, but, contrary to other non-steroidal anti-inflammatory drugs (NSAID) like indomethacin, does not influence PGI_2 formation by rat gastric mucosa *ex vivo* and is not ulcerogenic. The authors concluded that some NSAID can selectively inhibit PG biosynthesis in different tissues *in vivo* and, furthermore, that there is a relationship between the production of gastric erosions and inhibition of gastric mucosal PGI_2 formation by NSAID. We have further investigated the tissue selectivity of BW755C by comparing its effects on PGI_2 synthesis in three different regions of rat stomach, forestomach, corpus mucosa and antrum mucosa, both *in vitro* and *ex vivo*.

Materials and methods

In the *in vitro* experiments whole cell preparations of gastric tissue were prepared as described by Knapp *et al.* [3] (90 mg wet wt/incubate for forestomach and corpus mucosa, 30 mg/incubate for antrum mucosa). Mucosal tissue of corpus and antrum was separated from the underlying smooth muscle layer, while the forestomach preparation consisted of the whole stomach wall. The tissues were washed in ice-cold Krebs–Henseleit bicarbonate buffer [3] and then incubated in the absence or presence of various concentrations (10^{-5}–10^{-3} mol/l) of BW755C at 37° for 10 min. BW755C had been obtained from Wellcome Research Labs. (Beckenham, U.K.) and was freshly dissolved before use. In addition, incubations were performed with indomethacin (10^{-7}–10^{-3} mol/l; Merck, Sharp & Dohme, Rahway, NJ) as a standard PG synthesis inhibitor [4]. Under the experimental conditions used PG release by gastric tissue was linear for at least 10 min. At the end of the incubation period the medium was removed and aliquots were analysed for their content of 6-keto-$PGF_{1\alpha}$, the stable hydration product of PGI_2, using a highly sensitive and specific radioimmunoassay [5].

In the *in vivo* experiments BW755C (100 mg/kg) or indomethacin (2.5 mg/kg), suspended in 0.25% (w/v) carboxymethylcellulose, were administered orally to rats (1.0 ml/kg). Controls received the solvent only. Groups of rats were killed 30 min or 180 min after administration of BW755C and 180 min after administration of indomethacin. Tissue of the forestomach, corpus mucosa and antrum mucosa was rapidly isolated, washed in ice-cold Krebs–Henseleit bicarbonate buffer and incubated as whole cell preparations at 37° for 10 min [3]. The amount of 6-keto-$PGF_{1\alpha}$ released into the incubates was determined as

Table 1. Inhibition of synthesis of 6-keto-$PGF_{1\alpha}$ by tissue from three different regions of rat stomach by indomethacin and BW755C *in vitro**

Gastric region	Indomethacin IC_{50} (x 10^{-5} mol/l)	BW755C IC_{50} (x 10^{-5} mol/l)
Forestomach	3.2 ± 1.2	2.0 ± 0.6
(*n*)	(8)	(4)
Corpus mucosa	3.7 ± 1.5	20.8 ± 4.7
(*n*)	(8)	(4)
Antrum mucosa	4.7 ± 1.3	18.0 ± 4.7
(*n*)	(8)	(6)

* Results are the mean ± S.E.M. derived from *n* dose–response curves.