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Measurement ex-vivo of the inhibition of fatty acid biosynthesis by bezafibrate administration in different rat tissues

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The action of bezafibrate on fatty acid biosynthesis pathways has been examined in rat tissues. The drug abolished the induction of fatty acid synthesis that occurs on refeeding the animals with a high carbohydrate diet and by insulin administration in liver and adipose tissue. The rates of fatty acid biosynthesis in these tissues were estimated from the incorporation of ^3H into lipids from ^3H_2O.

Bezafibrate decreases the levels of triglycerides and cholesterol in animals (Berndt et al 1978) and is effective in lowering elevated levels of VLDL and LDL in patients with hyperlipoproteinaemia (Arntz et al 1980; Bolzano et al 1980; Olsson 1980). However, its specific mode of action is not yet understood.

The action of drugs on lipid metabolism may be of two kinds: (i) action on catabolic pathways and (ii) action on biosynthetic pathways. Bezafibrate increases the catabolism of lipids by producing a rapid increase in the activity of the peroxisomal β-oxidation system (Lazarow et al 1982). This supports the hypothesis (Lazarow 1977, 1978) that the mechanism of action of hypolipidaemic drugs involves an increased oxidation of fatty acids in hepatic peroxisomes. We have examined the effect of bexafibrate on fatty acid biosynthesis studied ex-vivo in those rat tissues that carry out this biosynthesis.

Materials and Methods

Female Wistar rats, 150–200 g, were placed in four groups: (i) rats described as fasted-refed were deprived of food for 48 h and then allowed free access for the next 48 h to a carbohydrate-rich diet; (ii) rats were treated with insulin for 3 days (8 units/100 g day^{-1}, subcutaneously); (iii) rats were fasted-refed and treated with bezafibrate during the refeeding period (150 mg kg^{-1} day^{-1} by gastric intubation); (iv) rats were simultaneously treated with insulin (8 units/100 g day^{-1}) and with bezafibrate (150 mg kg^{-1} day^{-1}) for 3 days.

All chemicals used were of the highest purity available. Insulin was from Nordisk Gentofte (Denmark). Bezafibrate was a gift from Laboratorios Andreu (Spain). Tritiated water was purchased from Radiochemicals Centre (Amersham, UK).

For the preparation of tissue extracts, rats were killed by cervical dislocation and the liver and adipose tissue rapidly removed and homogenized in 7 volumes of ice-cold medium containing 0·25 M sucrose, 1 mM EDTA, 1 mM dithiothreitol and 15 mM Tris-HCl, pH 7·4. This was centrifuged at 800g for 10 min.

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Glucose-6-phosphate dehydrogenase activity was determined as described by Sapag-Hagar et al (1973). Proteins were determined by the method of Lowry et al (1951) with bovine serum albumin as standard.

Fatty acid synthesis was estimated by monitoring the incorporation of ^3H_2O into fatty acids of liver and adipose tissue after intraperitoneal injection of ^3H_2O. Rats were injected intraperitoneally with 5 mCi of ^3H_2O. After 50 min, rats were anaesthetized with pentobarbitone (Nembutal, 50 mg kg^{-1}) and 10 min later, samples of tissue (1 g) or heparinized blood were added to 3 mL of 30% (w/v) KOH and the lipids saponified and extracted by the method of Stansbie et al (1976). The rate of fatty acid synthesis was calculated as μmol of ^3H_2O incorporated $h^{-1}g^{-1}$ wet wt of tissue and the specific radioactivity of plasma water measured in blood samples taken at the same time as tissue samples.

Results and discussion

Two metabolic conditions which increase the lipogenic capacity of rats, fasting-refeeding with a high carbohydrate diet and insulin administration (Young et al 1964; Timmers & Knittle 1980; Alexander et al 1982; Zammit & Corstorphine 1982), have been used to study the effect of bezafibrate on fatty acid biosynthesis in rat liver and adipose tissue. In both conditions without the drug there was a marked stimulation of fatty acid synthesis in liver and adipose tissue. Increases of 5- and 7-fold respectively were observed in these tissues of refed rats, whereas insulin treatment caused an increase of 8-fold in liver and 11-fold in adipose tissue (Table 1). At the same time, there was also an increase of newly synthesized fatty acids circulating in blood by nearly 5-fold. In addition, parallel changes in the specific activity of hepatic glucose-6-phosphate dehydrogenase (a lipogenic enzyme which generates a significant portion of the NADPH required for the reduction step of fatty acid synthesis) were found.

After the administration of bezafibrate at the same time as refeeding or in conjunction with insulin administration, the results show that bezafibrate abolished the induction of fatty acid synthesis produced by diet and insulin (Table 1). Bezafibrate's effect was greater in refed rats, where the rate of fatty acid synthesis was diminished to less than 25% of the rate seen in controls. Bezafibrate's effect on fatty acid in blood was unclear but a clearance from plasma affected by the drug cannot be excluded.

Table 1. Effect of bezafibrate on the rate of fatty acids synthesis in liver and adipose tissue, on radioactivity incorporated into circulating fatty acids newly synthesized from the liver, and on hepatic glucose-6-phosphate dehydrogenase activity. All animals were injected intraperitoneally with ^3H_2O (5 mCi/0·5 mL). After 1 h, samples of tissue (1 g) were removed. The incorporation of ^3H into tissue fatty acids and specific activity were determined as described in Materials and Methods section. The results are means ± s.e.m. for the number of animals shown in parentheses. $^aP < 0·05$; $^bP < 0·025$; $^cP < 0·0125$; $^dP < 0·01$; $^eP < 0·005$; $^fP < 0·0025$; $^hP < 0·0005$; * compared with control; ** compared with fasted-refed animals; *** compared with values for insulin-treated rats.

Treatment	μmol of ^3H_2O incorporated into fatty acids h^{-1} g^{-1} wet wt of tissue			glucose-6-phosphate dehydrogenase activity in liver (nmol min^{-1} mg^{-1} of protein)
	Liver	Adipose tissue	Blood	
Control	10·49 ± 1·30 (4)	6·35 ± 0·87 (4)	0·82 ± 0·05 (4)	54·5 ± 1·9 (18)
Fasting-refeeding	52·09 ± 10·82 ^{d*} (4)	43·05 ± 2·77 ^{f*} (4)	4·33 ± 1·83 ^{b*} (4)	104·8 ± 7·8 ^{h*} (11)
Fasting-refeeding + bezafibrate	2·51 ± 0·15 ^{c*} (4) ^{b**}	5·01 ± 1·31 ^{f**} (4)	0·87 ± 0·47 ^{a**} (4)	60·0 ± 2·4 ^{d**} (4)
Insulin	78·45 ± 16·16 ^{d*} (4)	67·52 ± 5·75 ^{f*} (4)	3·38 ± 1·03 ^{b*} (4)	131·4 ± 9·1 ^{h*} (6)
Insulin + bezafibrate	18·95 ± 2·21 ^{c*} (4) ^{c***}	4·86 ± 0·37 ^{f***} (4)	1·06 ± 0·18 ^{a***} (4)	56·5 ± 3·7 ^{h***} (4)

The present results suggest that this inhibition of fatty acid biosynthesis by bezafibrate measured ex-vivo could be mainly caused by a specific action on biosynthesis rather than indirectly related to increases in lipid catabolism since: (i) treatment with bezafibrate over 2 or 3 days does not produce a large induction of peroxisomal β-oxidation (Harrison 1984); (ii) peroxisomal β-oxidation is mainly involved in the chain shortening of long chain fatty acids (e.g. C_{22}) which are poor substrates for mitochondria (Christiansen 1978; Christiansen et al 1979; Osmundsen et al 1980); (iii) fatty acid oxidation by mitochondria is reduced in lipogenic conditions – fasting-refeeding and insulin treatment (McGarry & Foster 1980); (iv) in these conditions, bezafibrate treatment could produce a variation in the proportion of long chain fatty acids to short chain fatty acids, but this would not change the measurement of the rate of fatty acid biosynthesis using tritiated water, due to the fact that all fatty acids are extracted; (v) bezafibrate also abolishes the induction of hepatic glucose-6-phosphate dehydrogenase, considered as belonging to the family of lipogenic enzymes (Miksicek & Towle 1982). This effect could have another implication, because it could be due to the fact that NADPH, produced by this enzyme, is not required for fatty acid synthesis, since this process is inhibited.

From these results, we may conclude that the action of bezafibrate on fatty acid biosynthesis could make a contribution to its lipid lowering effect.

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