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MECHANISMS RESPONSIBLE FOR THE INHIBITORY EFFECTS OF BENFLUOREX ON HEPATIC INTERMEDIARY METABOLISM

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Abstract—The effects of benfluorex on hepatic intermediary metabolism have been studied using the isolated hepatocyte system. The drug inhibits the synthesis of both glucose and fatty acids by hepatocytes. Evidence is obtained that hepatocytes rapidly split benfluorex into benzoic acid and 1-(3-trifluoromethylphenyl)-2-[*N*-(2-hydroxyethyl)amino]propane (THEP). Comparison of the effects of the parent compound with the effects of THEP and benzoic acid on gluconeogenesis and on fatty acid synthesis indicates that different metabolites of the drug are responsible for its various actions: THEP inhibits gluconeogenesis, whereas benzoic acid inhibits fatty acid synthesis. The latter pathway appears to be inhibited at two sites: mitochondrial pyruvate uptake is inhibited by benfluorex itself, whereas fatty acid synthase is inhibited by benfluorex-derived benzoic acid.

In the course of our studies on hormonal regulation of hepatic lipogenesis we became interested in specific inhibitors of fatty acid synthesis. Such compounds are of considerable interest because they are potential tools in studies on metabolic regulation and can possibly be used as hypolipidemic drugs (cf. refs. [1–3]).

Benfluorex, 1-(3-trifluoromethylphenyl)-2-[*N*-(2-benzyloxy-ethyl)amino]propane, is a relatively new hypolipidemic agent. Evidence has been presented that the compound affects hepatic lipid metabolism. Feeding of benfluorex to rats decreases their serum-triacylglycerol level [4]. Since the liver is an important, if not the most important, site for the production of serum triacylglycerols, it is likely that the hypolipidemic action of benfluorex is exerted at the level of hepatic lipogenesis. Indeed, Brindley and Bowley [5] reported suppression of phosphatidate phosphohydrolase (EC 3.1.3.4) activity in benfluorex-treated homogenates of rat liver. In addition, the drug has been shown to inhibit *de novo* fatty acid synthesis by isolated hepatocytes [6].The biochemical basis of the latter action is not clear.

The present experiments were mainly designed to elucidate the effects of benfluorex on hepatic fatty acid biosynthesis. Attention has been focused on the acute effects of the drug for the following reasons. First, if the drug has potential as a tool in biochemical studies it will be of most interest in short-term manipulation of metabolism. Secondly, although the chronic effects of hypolipidemic drugs are the most important from a therapeutic point of view, their daily administration will cause the patient's lipid metabolism on the short term to oscillate due to fluctuations in the drug concentration of the blood.

Parts of this study have been presented in preliminary form [7].

MATERIALS AND METHODS

Isolation and incubation of hepatocytes. Hepatocytes were isolated from male Wistar rats (225–250 g), which had free access to water and were meal-fed a stock, pelleted diet between 4 a.m. and 7 a.m. by an automatic feeding machine. The animals were sacrificed at 9 a.m. Liver cells were isolated according to Seglen [8], with modifications described previously [9]. Cell viability (trypan blue exclusion) was routinely better than 95%. In previous studies these cells had been shown to be hormone-sensitive with regard to *de novo* fatty acid synthesis [9]. This indicates that the hepatocytes are suitable for metabolic studies (cf. ref. [10]). Isolated hepatocytes (3–6 mg protein/ml) were suspended in Krebs–Henseleit bicarbonate buffer, supplemented with 3.5% bovine serum albumin (charcoal-treated and dialysed) and 10 mM glucose. Incubations (final vol. 3 ml) were carried out at 37° in a metabolic shaker (90 strokes/min) in 25-ml Erlenmeyer flasks under an atmosphere of 95% oxygen and 5% carbon dioxide.

Isolation and incubation of mitochondria. For the preparation of mitochondria, livers from female Wistar rats (125–150 g) were homogenised in 250 mM sucrose, 5 mM Tris–HCl (pH 7.50) and 1 mM EGTA. Mitochondria were isolated as described by Myers and Slater [11], suspended in the same medium without EGTA and stored at 0°. The incubation medium was composed of 12.5 mM sucrose, 15 mM KCl, 5 mM $MgCl_2$, 0.05 mM EGTA, 2 mM EDTA, 50 mM Tris–HCl (pH 7.50), 10 mM succinate, 5 mM phosphate and 6 μg rotenone.

Assay of oxygen consumption. The rate of oxygen consumption was followed with a Clark oxygen electrode in a closed reaction vessel. The incubation medium (2 ml) contained 3–4.5 mg of mitochondrial

protein or 4–5 mg of cellular protein. In the studies with mitochondria the final concentration of ADP, when added, was 0.15 mM. The term State-4 respiration refers to the terminology according to Chance and Williams [12] and indicates that the supply of ADP is rate-limiting for respiration and oxidative phosphorylation.

Glucose and lipid synthesis; triacylglycerol secretion. The rate of gluconeogenesis was determined using hepatocytes from 24-hr starved rats incubated for 60 min with either 10 mM lactate plus 1.5 mM pyruvate or with 10 mM alanine in the absence or presence of the various compounds under study. Incubations were terminated with $HClO_4$ (final concentration 0.5 M) and glucose was assayed by the GOD–Perid method.

To monitor rates of fatty acid synthesis, ^3H_2O (0.3 mCi/ml) was added to the cell suspensions. Incubations were terminated by addition of $HClO_4$ (final concentration 0.5 M). Fatty acids were extracted according to Harris [13] and Kates [14].

To study the secretion of triacylglycerols, hepatocyte triacylglycerols were prelabelled by incubating the cells (no drugs present) in bicarbonate buffer (10 mM glucose, 3.5% albumin) containing ^3H_2O (2 mCi/ml). After 1 hr the cells were washed twice by centrifugation at room temperature for 1 min at 100 g and resuspension of the pellet (intact hepatocytes) in radioisotope-free bicarbonate buffer (10 mM glucose, 3.5% albumin). The hepatocytes were then re-incubated for an additional 1 hr in the absence or presence of the compound under investigation. At the end of the second incubation period cells were separated from the medium by spinning through silicone oil as described before [15]. The appearance of prelabelled triacylglycerols in the supernatant was determined as described previously [15].

Enzyme assays. For measuring the effects of drugs on enzyme activities partially purified preparations of acetyl-CoA carboxylase (EC 6.4.1.2) [16], fatty acid synthase [17] and malic enzyme (EC 1.1.1.40) [18] were prepared from rat-liver homogenates by ammonium sulphate precipitation, column chromatography and electrophoresis. Glucose 6-phosphate dehydrogenase (EC 1.1.1.49) and 6-phosphogluconate dehydrogenase (EC 1.1.1.44) were purchased. Following a 15-min preincubation in the presence or absence of benfluorex or benzoic acid, enzyme activities were determined according to refs. [16–20] for acetyl-CoA carboxylase, fatty acid synthase, malic enzyme, glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase, respectively.

Assays of metabolites; protein determination. Metabolite assays were conducted on KOH-neutralized $HClO_4$ extracts of hepatocytes according to the methods of Hohorst [21] for pyruvate and lactate, Passonneau and Brown [22] for citrate, Williamson *et al.* [23] for acetoacetate and 3-hydroxybutyrate, and Hassid and Abraham [24] for glycogen.

Protein was determined by the Lowry procedure [25].

Sources of materials. Radioactive compounds were obtained from the Radiochemical Centre, Amersham, U.K.; collagenase type I and bovine serum albumin were purchased from Sigma; other enzymes, GOD–Perid kits and biochemicals were from Boehringer; most other chemicals were from Baker; sodium benzoate was obtained from Merck; benfluorex and 1-(3-trifluoromethylphenyl)-2-[*N*-(2-hydroxyethyl)amino]propane (THEP) were gifts from Servier, Paris.

RESULTS

Effect of benfluorex on fatty acid synthesis monitored with ^3H_2O

Bicarbonate-buffered suspensions of hepatocytes were incubated in the presence of 10 mM glucose as the sole exogenous carbon source and of benfluorex at various concentrations. The incorporation of ^3H_2O into fatty acids was determined over the 0–60 min incubation period. The ^3H_2O method is considered the most reliable method available to assess rates of fatty acid synthesis [26]. Figure 1 documents the inhibitory effect of benfluorex on the rate of fatty acid synthesis. At a concentration of approximately 0.28 mM benfluorex reduces fatty acid synthesis to 50% of the control value.

Effect of benfluorex on metabolite levels of hepatocytes.

The rate of *de novo* fatty acid synthesis by isolated rat hepatocytes is strongly influenced by the flux of glucose carbon through the glycolytic pathway [13]. On the other hand, glycogen synthesis may compete with glycolysis for their mutual substrate glucose. Since the rate of glycogenesis is dependent on the cellular level of glycogen, the latter may indirectly affect the rate of lipogenesis. For these reasons, the effect of benfluorex on glycolysis and on glycogen levels was investigated. Neither the concentration of glucose in the cell suspension nor the cellular glycogen content are affected to any appreciable extent by the presence of the drug (Table 1). This indicates that the drug does not divert glucose from glycolysis to glycogenesis. Glycolysis, on the other hand, seems to be influenced by incubation of hepatocytes with benfluorex. Both pyruvate and lactate levels are increased in the presence of benfluorex (Table 1). The increase in lactate is much more marked. In the presence of benfluorex, NAD in the cytoplasm was more reduced (lactate/pyruvate ratio increased), perhaps reflecting the rate of utilization of reducing equivalents caused by benfluorex inhibition of fatty acid synthesis. On the other hand, mitochondrial NAD, as indexed by the 3-hydroxybutyrate/acetoacetate ratio, was more oxidized in the presence of benfluorex. This compound, therefore, appears to cause a defect in the transport of reducing equivalents from the cytosol to the mitochondria. This defect cannot be responsible for inhibition of fatty acid synthesis by benfluorex, because an excess of reducing equivalents in the cytosol will favor fatty acid synthesis.

Effect of benfluorex on mitochondrial pyruvate uptake

The increased accumulation of pyruvate plus lactate (Table 1) in the cell suspension following a 60 min incubation period in the presence of benfluorex points to a site of drug action at the level of

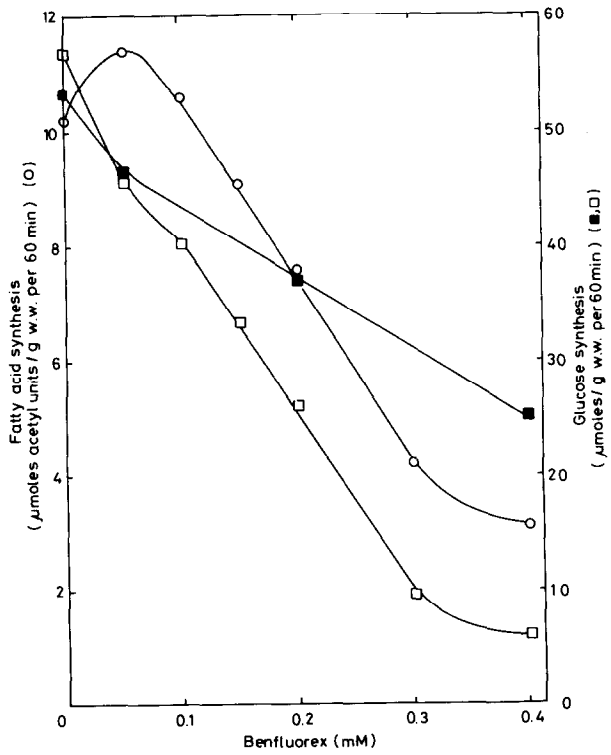

Fig. 1. Effects of benfluorex on fatty acid synthesis and gluconeogenesis. Fatty acid synthesis (○) by hepatocytes from fed rats was measured by ^3H_2O incorporation into fatty acids. Gluconeogenesis from lactate plus pyruvate (□) or from alanine (■) by hepatocytes from 24-hr starved rats was monitored by measuring net glucose production.

pyruvate utilization. Pyruvate dehydrogenase (EC 1.2.4.1) activity and/or mitochondrial pyruvate uptake (via the mitochondrial pyruvate carrier) are possible candidates for such a site.

The activity of pyruvate dehydrogenase, measured following incubation of isolated mitochondria in the presence of benfluorex, is unaffected by the drug (data not shown).

With increasing concentrations of benfluorex, uptake of [2-^{14}C]pyruvate into isolated rat-liver mitochondria is increasingly inhibited (Fig. 2), the inhibition being 50% at approximately 0.4 mM. This observation most likely explains the observed increase in pyruvate plus lactate accumulation of the total cell suspension in the presence of benfluorex (Table 1). In other words, the action of benfluorex

Table 1. Effects of benfluorex on metabolite levels, metabolite ratios and rates of metabolite formation in suspensions of hepatocytes from meal-fed rats

	Control	Benfluorex
Glycogen content*	285	276
Glucose production†	18	20
Lactate level‡	114	168
Pyruvate level‡	41	47
Lactate/pyruvate ratio	2.76	3.54
Lactate + pyruvate accumulation§	56	94
Acetoacetate level‡	61	48
3-Hydroxybutyrate level‡	16	8
3-Hydroxybutyrate/acetoacetate ratio	0.27	0.16
3-Hydroxybutyrate + acetoacetate accumulation§	49	28
Citrate level‡	8.6	8.4

* μg/mg protein.
† μg/mg protein per hr.
‡ nmoles/mg protein.
§ nmoles/mg protein per hr.
Hepatocytes were incubated for 60 min in the absence or presence of 0.4 mM benfluorex. Results are expressed as means of two preparations of hepatocytes.

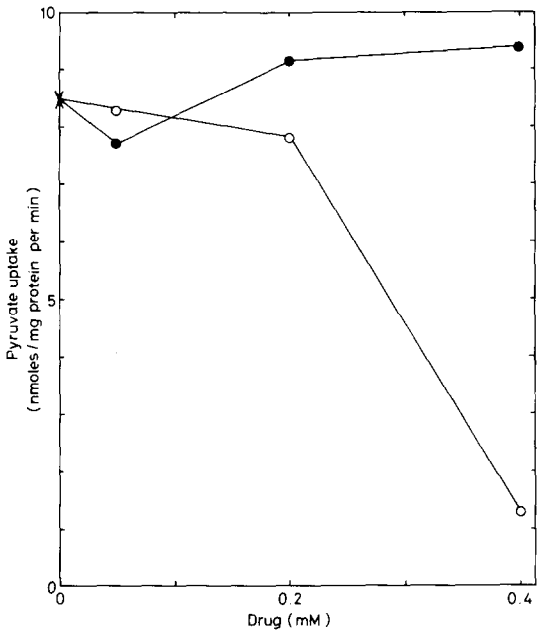

Fig. 2. Mitochondrial accumulation of [2-^{14}C]pyruvate as a function of benfluorex and benzoic acid concentration. (○) Pyruvate uptake in the presence of benfluorex, (●) in the presence of benzoate. Pyruvate uptake was determined as described before [41].

in this respect is comparable to the action of α-cyano-4-hydroxycinnamate, a well-recognized inhibitor of mitochondrial pyruvate uptake [27].

Inhibition of pyruvate transport, however, cannot be the sole cause of benfluorex inhibition of fatty acid synthesis since this process is also inhibited when acetate is used as precursor for lipogenesis [6]. This argues for (an) additional site(s) of action, different from pyruvate translocation and present in that part of the lipogenic pathway responsible for the cytosolic conversion of acetate into fatty acids.

Effect of benfluorex on gluconeogenesis

Gluconeogenesis from lactate plus pyruvate and from alanine by hepatocytes isolated from 24-hr starved rats is progressively inhibited by increasing concentrations of benfluorex, the former process being more sensitive to the drug (Fig. 1). Inhibition of gluconeogenesis by 50% required 0.18 mM benfluorex with lactate plus pyruvate and 0.30 mM with alanine as the gluconeogenic precursor.

Effect of benfluorex on energy metabolism

Both lipogenesis and gluconeogenesis are energy-requiring processes. Benfluorex may interfere with the synthesis of fatty acids and glucose by limiting the energy supply. Therefore, the effect of benfluorex on oxidative phosphorylation in mitochondria prepared from rat liver was studied. Benfluorex appears to uncouple State-4 respiration by isolated mitochondria (data not shown). This is indicated by an increased ratio of the rates of oxygen consumption in the presence and absence of the drug under State-4 conditions. Half-maximal stimulation of State-4 respiration occurs at about 0.05 mM benfluorex.

The question arises whether uncoupling of oxidative phosphorylation is also of significance in intact hepatocytes at concentrations of benfluorex which inhibit lipogenesis and gluconeogenesis by these cells. The data (not shown) suggest that oxidative phosphorylation in intact cells is not significantly affected by drug treatment. Evidence that the concentrations of benfluorex employed (0.05–0.40 mM) do not impair oxidative phosphorylation in intact hepatocytes comes from the observation that these concentrations of the drug do not influence the cellular level of ATP (data not shown).

Evidence that benfluorex is converted to active metabolites by isolated rat hepatocytes

Since there is no indication that benfluorex causes uncoupling of oxidative phosphorylation in intact cells, it follows that the concentration of free benfluorex within the intact cell probably never reaches a level which can uncouple oxidative phosphorylation. Perhaps benfluorex in the cell is metabolized in such a way that the resulting compound(s) does not affect oxidative phosphorylation but does impair fatty acid synthesis and gluconeogenesis. This possibility was examined. It has been reported that the drug is completely converted to 1-(3-tri-fluoromethylphenyl) - 2 - [*N* - (2 - hydroxyethyl) amino] propane (THEP) (Fig 3) on incubation with fortified liver homogenates of guinea pig [28]. In the course of such a metabolic conversion equimolar amounts of benzoic acid must be formed. Due to the abundance of esterases in rat liver, it is likely that also in intact rat-liver cells the ester bond in the drug molecule is split, releasing benzoic acid and THEP. Therefore, the effect of these drug metabolites on fatty acid synthesis and on gluconeogenesis (Table 2) was compared with the effect of the parent compound, benfluorex. The results indicate that the effect of benfluorex on hepatic fatty acid synthesis can be mimicked by benzoic acid, whereas THEP mimics the effect of the parent compound on gluconeogenesis (Table 2).

The evidence presented in Table 2 was suggestive, but did not prove that benfluorex was actually split by hepatocytes into benzoic acid and THEP. Definitive evidence was obtained by analysing the absorption spectra of benfluorex, benzoic acid and THEP. Benfluorex has absorption maxima at 263.5 and 270.5 nm, whereas THEP absorbs maximally at 262.5 and 269.5 nm and benzoate has no maximum at all. Within 5 min the absorption spectrum of benfluorex disappears completely upon incubation of the drug with liver cells. At the same time an absorption

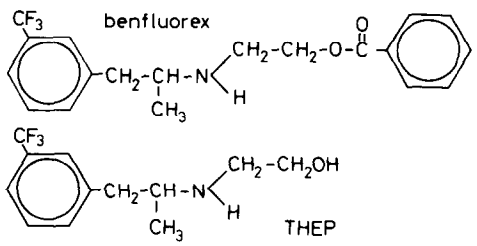

Fig. 3. Chemical structures of compounds studied.

Table 2. Effects of benfluorex, benzoic acid and THEP on glucose and fatty acid synthesis in hepatocytes from 24-hr starved and fed rats, respectively

Additions (mM)	Gluconeogenesis (nmoles glucose/mg protein per hr)			Fatty acid synthesis (nmoles acetyl units/mg protein per hr)		
	Benfluorex	Benzoate	THEP	Benfluorex	Benzoate	THEP
0	210.0	—	—	37.7	—	—
0.05	168.5	192.9	167.4	42.2	37.4	39.6
0.10	148.9	195.9	142.2	39.2	35.9	40.4
0.15	123.3	197.4	126.3	33.7	34.1	39.2
0.20	96.3	198.5	108.5	28.1	31.1	39.2
0.30	35.2	192.9	81.1	15.5	22.2	39.2
0.40	22.2	187.4	62.6	11.5	11.1	36.7

For gluconeogenesis lactate and pyruvate were present at initial concentrations of 10 and 1.5 mM, respectively. Fatty acid synthesis was monitored by incorporation of tritium from ^3H_2O into fatty acids. Values are the means of triplicate incubations in a representative experiment.

spectrum characteristic for THEP is produced (Fig. 4). Thus, these experiments establish that liver cells are capable of metabolizing benfluorex to its active components, viz. benzoic acid and THEP.

Effect of benfluorex and benzoic acid on the activity of lipogenic enzymes

A series of experiments was undertaken to investigate whether the reduction in the rate of fatty acid biosynthesis resulting from the addition of benfluorex or benzoic acid and residing in the lipogenic pathway *per se* (see above), could be brought about by a direct inhibition of one or more of the enzymes involved in *de novo* fatty acid synthesis. Experiments were conducted with partially purified preparations of lipogenic enzymes. The enzymes studied are those

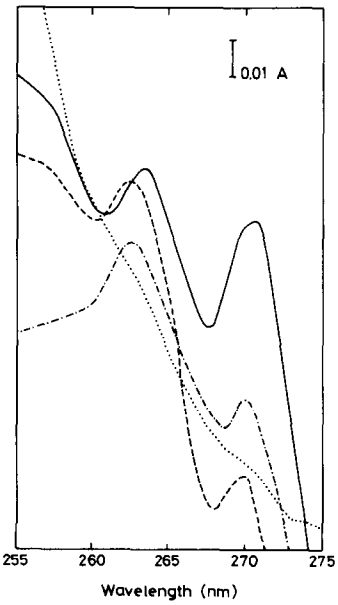

Fig. 4. Dual wavelength scans of the absorption spectrum from 255 to 275 nm are shown of benfluorex (—), THEP (---), benzoate (...) and of benfluorex incubated for 5 min with hepatocytes (-·-·-). Reference wave length: 260 nm. For the absorption maxima see text.

responsible for the generation of cytosolic reducing equivalents and for the conversion of cytosolic acetyl-CoA into palmitate. Table 3 compares the effects of benfluorex and benzoic acid on enzyme activities following a 15-min preincubation of the enzyme preparation with the compounds under investigation. Benfluorex was found to have no significant effects upon any of these enzyme activities. However, benzoic acid very effectively inhibited fatty acid synthase activity. Even without preincubation, benzoic acid completely inhibited this enzyme activity (data not shown). The data in Table 3 again indicate that benzoic acid is probably the active component of benfluorex as far as its action on fatty acid synthesis is concerned. Although both compounds inhibit fatty acid synthesis in intact hepatocytes, only benzoic acid has an effect on one of the enzyme activities essential for *de novo* fatty acid synthesis, viz. fatty acid synthase.

Time course of the inhibitory effect of benfluorex and benzoic acid on fatty acid synthesis

Since benzoic acid immediately and completely inhibits fatty acid synthase activity, whereas it inhibits only partially the rate of fatty acid synthesis by isolated hepatocytes, measured over the entire 0–60 min incubation period, it follows that benzoic acid is probably further metabolized to a compound which is less effective or even ineffective with respect to fatty acid synthesis. Indeed, when hepatocytes are incubated with benfluorex or benzoic acid the inhibitory effect of the drugs on the subsequently determined rate of fatty acid synthesis diminishes. Within 60 min both compounds have lost their potential to inhibit fatty acid synthesis (Fig. 5).

Effect of glycine on the benfluorex- and benzoic acid-inhibited rate of fatty acid synthesis

Further proof that benfluorex-derived benzoic acid is responsible for inhibition of fatty acid synthesis stems from experiments in which fatty acid synthesis was monitored in the presence of glycine. Since the liver forms hippurate from glycine and benzoic acid [29, 30], it was expected that glycine would more or less prevent the inhibition of fatty acid synthesis by

Table 3. Effects of benfluorex and benzoic acid on the activity of enzymes involved in *de novo* fatty acid synthesis

Enzyme	Enzyme activity (percentage of control)		
	Control	Benfluorex	Benzoic acid
Acetyl-CoA carboxylase	100	100	100
Fatty acid synthase	100	88	0
Glucose 6-phosphate dehydrogenase	100	100	100
6-Phosphogluconate dehydrogenase	100	100	100
Malic enzyme	100	100	100

Enzyme activities were determined following a 15-min preincubation of the enzyme preparations in the absence or presence of 0.4 mM of the drugs.

either benfluorex or benzoic acid. Glycine itself was found to cause a slight inhibition of fatty acid synthesis (Fig. 6). Nevertheless, glycine very strikingly counteracted the inhibitory effects of benfluorex and benzoic acid on fatty acid synthesis, suggesting that hippurate formation prevented benzoic acid from continuously and effectively inhibiting fatty acid synthesis (Fig. 6).

Effect of benfluorex on triacylglycerol secretion by isolated hepatocytes

Feeding of benfluorex to rats results in a decrease in serum triacylglycerols. Inhibition of hepatic fatty acid synthesis by benfluorex will impair triacylglycerol synthesis. The process of fatty acid esterification itself, however, is not impaired at the highest concentration of benfluorex tested in this study, i.e. 0.4 mM. This notion is derived from the observation that triacylglycerol synthesis from [1-^{14}C]oleate is unaffected by addition of 0.4 mM benfluorex to cell suspensions (data not shown). Since the inhibition of fatty acid synthesis by benfluorex is transient (Fig. 5), the question arises whether this inhibition is solely responsible for the lipid-lowering effect of the drug or whether additional sites in the total process of serum-lipid formation are affected. Therefore, the effect of benfluorex on triacylglycerol secretion was studied. Triacylglycerol secretion was measured independently from triacylglycerol synthesis. This was achieved by prelabelling hepatocyte triacylglycerols with ^{3}H from $^{3}H_2O$. Following removal of $^{3}H_2O$, triacylglycerol secretion was monitored as the appearance of these prelabelled triacylglycerols in the medium. The data in Fig. 7 illustrate that, apart from fatty acid synthesis, also triacylglycerol secretion is markedly impaired by benfluorex. With increasing drug concentrations secretion of triacylglycerols is progressively inhibited.

The inhibition of hepatic fatty acid synthesis, albeit transient, may co-operate to counteract the occurrence of fatty liver in the face of inhibition of triacylglycerol secretion.

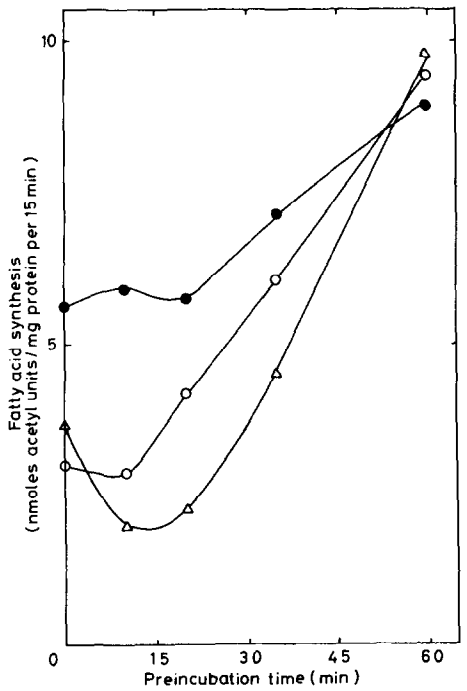

Fig. 5. Time course of the inhibition of fatty acid synthesis in hepatocytes by benfluorex and benzoic acid. Hepatocytes were preincubated for the indicated periods of time in the absence or presence of benfluorex and benzoic acid. Following the preincubation period the rate of fatty acid synthesis was monitored for 15 min with $^{3}H_2O$ (1.33 mCi/ml). Control (●), 0.4 mM benfluorex (○) and 0.4 mM benzoic acid (△).

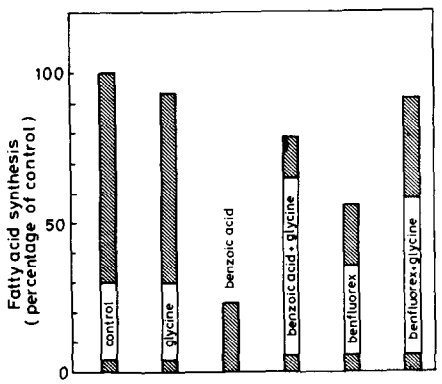

Fig. 6. Effect of glycine on the inhibition of fatty acid synthesis by benfluorex and benzoic acid. Hepatocytes were incubated for 60 min with $^{3}H_2O$ (0.3 mCi/ml) in the absence or presence of the drugs indicated. In addition, some incubations contained 2 mM glycine.

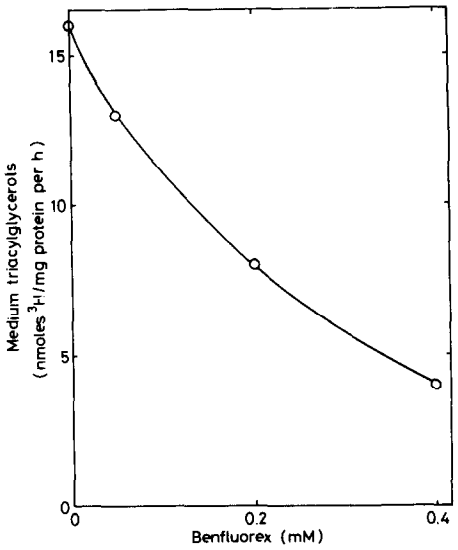

Fig. 7. Dose-response curve of the effect of benfluorex on the release of prelabelled triacylglycerols. Hepatocytes previously incubated for 60 min with ^3H_2O (2 mCi/ml; no drug present), were washed and further incubated for 60 min in a radioisotope-free buffer in the absence or presence of various drug concentrations.

DISCUSSION

This study shows that benfluorex inhibits both fatty acid and glucose synthesis by isolated rat hepatocytes. Evidence is provided that hepatocytes rapidly convert benfluorex into THEP and benzoic acid. The latter compound is then further metabolized yielding hippuric acid [29, 30]. Comparison of the effects of THEP and benzoic acid with the effects of benfluorex on gluconeogenesis and fatty acid synthesis indicates that these drug metabolites may be responsible for some of the actions attributed to benfluorex. Furthermore, as noted before [29], fatty acid synthesis is markedly more sensitive to benzoic acid inhibition than glucose synthesis. This suggests that some compound different from benzoic acid may be responsible for inhibition of the latter process. Indeed, THEP, an established metabolite of benfluorex [28], was found to impair gluconeogenesis approximately to the same extent as the parent compound, whereas benzoic acid at the same concentration hardly affected this process. It is proposed that the two products which result from the hydrolysis of benfluorex are responsible for different parts of the metabolic action of benfluorex, viz. THEP or a further metabolite represents the effect of benfluorex on gluconeogenesis and benzoic acid or one of its metabolites stands for the action of benfluorex on fatty acid biosynthesis.

The metabolism of benzoic acid by isolated hepatocytes results in the formation of hippurate [29, 30]. Hippurate synthesis requires an activated form of benzoic acid which appears to be benzoyl-CoA [31]. A number of compounds which form CoA esters have been demonstrated to inhibit various metabolic processes of the liver either by sequestration of CoA or by formation of inhibitory CoA esters from the parent compound or from a metabolite [32–39]. However, since hippurate formation from benzoic acid is associated with the mitochondria [40], it is unlikely that CoA sequestration or a direct action of benzoyl-CoA is involved in the effect of benzoic acid on the cytosolic process of fatty acid synthesis. Furthermore, if benzoyl-CoA inhibits fatty acid synthesis, this effect has to be transient because it is observed that with time the inhibition of fatty acid synthesis by benzoic acid, or for that matter by benfluorex, diminishes.

Apart from the effect of benfluorex-derived benzoate on fatty acid synthesis, via inhibition of fatty acid synthase activity, there is also an effect by benfluorex itself on the rate of fatty acid synthesis, i.e. on the conversion of glucose into fatty acids. Neither benzoate, which does not impair pyruvate uptake by isolated mitochondria (Fig. 2), nor THEP, to which fatty acid synthesis is insensitive (Table 2), are responsible for the effect of benfluorex on mitochondrial pyruvate uptake. This action of benfluorex (in isolated cells and probably also *in vivo*) also has to be transient since benfluorex is rather rapidly metabolized (Fig. 4).

Benfluorex inhibition of gluconeogenesis, from either pyruvate plus lactate or from alanine, is probably also exerted at the level of the mitochondrial pyruvate carrier, which is inhibited by benfluorex (Fig. 2). Whether inhibition of gluconeogenesis by THEP occurs at the same site, is unknown at present.

In summary, this study offers an explanation for the short-term effects of benfluorex on hepatic intermediary metabolism. It is concluded that benfluorex is metabolized by liver tissue in such a way that the two resulting compounds are each responsible for a different metabolic action of benfluorex, viz. benzoic acid inhibits fatty acid synthesis whereas THEP diminishes gluconeogenesis. In addition, benfluorex itself inhibits fatty acid synthesis and also gluconeogenesis by inhibition of mitochondrial pyruvate uptake. The results indicate that benfluorex may be useful in the treatment of diabetes since the compound counteracts several abnormalities observed in diabetes, viz. increased serum concentrations of glucose, ketone bodies and triacylglycerols. Details concerning the molecular mechanisms of the inhibitory effects await further investigation.

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