Inhibition of liver microsomal carnitine acyltransferases by sulphonylurea drugs

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Abstract The sulphonylureas glibenclamide and tolbutamide inhibited carnitine acyltransferase activities in rat liver microsomes. Glibenclamide was a more potent inhibitor than tolbutamide. The effect of tolbutamide on the malonyl-CoA-inhibitable transferase was influenced by the phospholipid/detergent environment whereas the effect of glibenclamide was not. Glibenclamide was a more potent inhibitor of the malonyl-CoA-inhibitable transferase than of the malonyl-CoA-insensitive enzyme. The extent of inhibition of the malonyl-CoA-inhibitable transferase by tolbutamide was similar to its effect on VLDL triacylglycerol secretion as reported by Wiggins and Gibbons [Biochem. J. 284 (1992) 457–462] possibly supporting the suggestion that microsomal carnitine acyltransferases are involved in VLDL triacylglycerol assembly/secretion.

Key words: Carnitine acyltransferases; Glibenclamide; Liver; Microsome; Sulphonylureas; Tolbutamide

1. Introduction

Carnitine acyltransferases that catalyze the interconversion of medium- or long-chain fatty acyl-CoA esters with the corresponding acylcarnitines have been described in many mammalian tissues. In liver carnitine acyltransferase (CAT) activities have been reported in mitochondria, in peroxisomes, in microsomes and in nuclei [1–4]. Recently we [5] and others [6] have provided evidence that rat liver microsomes contain two distinct CATs. One of these activities is membrane-bound, appears to be oriented towards the cytosolic compartment and can be inhibited by physiological concentrations of malonyl-CoA. The other CAT activity is latent in 'intact' microsomes, is insensitive to malonyl-CoA and has a lumenal location. The physiological function of these microsomal CATs is not yet clearly established. It has been suggested [5] that they might be part of a system that enables transport of fatty acyl units between the cytosol and the lumen of the endoplasmic reticulum (this presupposes the existence also of a microsomal carnitine:acylcarnitine translocase). Studies with primary cultures of rat hepatocytes [7–9] have led to the conclusion that fatty acids incorporated into secreted VLDL triacylglycerols are largely derived from lipolysis of a hepatic cytoplasmic pool of triacylglycerol. As a corollary to this it would seem likely that the synthesis of these discrete pools of VLDL and cytoplasmic store triacylglycerols should be spatially separated and we have speculated [5] that provision of fatty acyl units to the lumen of the secretory compartment may be part of this separation. Wiggins and Gibbons [8] have shown that the sulphonylurea drug tolbutamide is able to suppress VLDL triacylglycerol secretion by hepatocytes. Tolbutamide appeared to have two actions. One was to decrease hepatocyte lipolysis. The other was an independent effect on subsequent VLDL assembly and/or secretion. Since some sulphonylurea drugs had previously been shown to inhibit carnitine acyltransferase activities from mitochondria [10] we decided to investigate whether tolbutamide and glibenclamide (glyburide) could inhibit one or other of the microsomal CATs.

2. Materials and methods

2.1. Chemicals

1-{4-[2-(5-chloro-2-methoxybenzamido)ethyl]benzene-sulphonyl}-3-cyclohexylurea (glibenclamide), 1-butyl-3-*p*-tolylsulphonylurea (tolbutamide) and *N*,*N*-dimethylformamide were from Sigma. Dimethylsulphoxide was from Fisons. Animals and all other reagents were as described previously [5].

2.2. Isolation of microsomal membranes

Rough microsomes were isolated from livers of fed male Sprague–Dawley rats (200–250 g) by sucrose density-gradient centrifugation [11]. Membrane residues depleted of malonyl-CoA-insensitive CAT were obtained by freeze/thawing and recentrifugation of membranes as described in [5].

2.3. Carnitine acyltransferases (CATs)

Malonyl-CoA-sensitive CAT was solubilized with sodium deoxycholate and partially purified by Superdex 200 gel-filtration [5]. Malonyl-CoA-insensitive CAT was released from intact microsomes by freeze-thawing/ultracentrifugation [5] and partially purified by ion-exchange chromatography on Resource Q [5].

2.4. Assay of carnitine acyltransferase

CAT activity was assayed spectrophotometrically at 25°C in the direction of acylcarnitine formation. Unless stated otherwise assays (1 ml) contained: 50 mM KH_2PO_4/K_2HPO_4 buffer (pH 7.5), 1.3 mg/ml fatty acid-poor bovine serum albumin, 125 μM 4,4′-dithiodipyridine, 100 μM decanoyl-CoA, 20–100 μg enzyme protein and the appropriate inhibitor. After 5 min incubation at 25°C assays were initiated by the addition of the stated concentration of L-carnitine. Rates were corrected for carnitine-independent CoASH release. Activity was calculated using $E_{324} = 19.4\ mM^{-1} \cdot cm^{-1}$. Glibenclamide was introduced into assays in 5 μl of dimethylformamide. Tolbutamide was introduced in 10 μl of dimethylsulphoxide. Controls (i.e. no inhibitor present) also received dimethylformamide or dimethylsulphoxide as appropriate.

2.5. Reconstitution of malonyl-CoA-sensitive CAT

Malonyl-CoA-sensitive CAT from the Superdex 200 column was reconstituted into L-α-lecithin liposomes exactly as described in [5].

2.6. Analysis of data

K_m and V_{max} values were determined using a computer programme in which starting values of K_m and V_{max} were obtained by linear regression fitting to a Hanes-Woolf plot and these values were then used to seed a non-linear regression analysis which fitted the data to the best fit hyperbola [12,13]. Statistically significance was determined by Student's *t*-test for paired samples.

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3. Results and discussion

Fig. 1 shows that both tolbutamide and glibenclamide inhibited the malonyl-CoA-sensitive CAT activity. With the enzyme in situ in membrane residues that had been frozen/thawed in order to deplete them of the malonyl-CoA-insensitive CAT approx. 2.1 mM tolbutamide caused 50% inhibition in assays with 100 μM carnitine (a concentration of this substrate which is not dissimilar from the concentration of free carnitine in rat liver [14,15]). Increasing the carnitine concentration to 2 mM lessened substantially the inhibitory effect of tolbutamide. With the concentration of the fatty acyl-CoA substrate decanoyl-CoA fixed at 100 μM (which is sufficient to saturate the enzyme) tolbutamide produced mixed inhibition kinetics with respect to carnitine (Table 1). We also examined the effect of tolbutamide on partially purified preparations of the malonyl-CoA-sensitive CAT. In detergent-solubilized form the CAT was less sensitive to inhibition by tolbutamide whereas reconstitution of the enzyme into lecithin liposomes more than restored 'normal' sensitivity to tolbutamide. In this respect it is noteworthy that sensitivity of this CAT to inhibition by malonyl-CoA is similarly affected by the detergent/phospholipid environment [5]. Fig. 1 also shows that approx. 30 μM glibenclamide caused 50% inhibition of the malonyl-CoA-sensitive CAT. By contrast with tolbutamide the inhibitory potency of glibenclamide was unaffected by the detergent/phospholipid environment. Furthermore variation in the concentration of carnitine caused no change in the sensitivity to glibenclamide (Fig. 1) and the effect of glibenclamide was clearly non-competitive with respect to carnitine (Table 1). The effects of the two drugs on the two distinct microsomal CATs were also compared (Fig. 2). Since the malonyl-CoA-insensitive enzyme can only be separated from the malonyl-CoA-sensitive CAT by solubilization procedures we decided to make the comparison using solubilized preparations of both enzymes. These differed only in that the malonyl-CoA-sensitive CAT was obtained from a Superose 200 gel-filtration in a buffer containing 5 mM sodium deoxycholate whereas the malonyl-CoA-insensitive enzyme was obtained from a Resource Q ion-exchange fractionation in the same buffer, but containing 1.5 mM sodium cholate. Tolbutamide had broadly similar inhibitory effects on both enzymes (Fig. 2)

Table 1
Effects of tolbutamide and glibenclamide on kinetic constants of the malonyl-CoA-sensitive CAT

Experiment no.	n	Sulphonylurea	V_{max} (relative)	K_m for L-carnitine (mM)
1	3	none	105 ± 1	047 ± 0.13
1	3	tolbutamide (5 mM)	81 ± 2[C]	1.08 ± 0.28[A]
2	3	none	105 ± 1	050 ± 0.06
2	3	glibenclamide (25 μM)	65 ± 4[B]	0.53 ± 0.10[NS]

All assays were performed with microsomal membrane residues which had been depleted of the malonyl-CoA-insensitive CAT by freezing/thawing. Throughout [decanoyl-CoA] was fixed at 100 μM and L-carnitine used at 6–7 different concentrations over the range 0.1 mM to 10 mM. Values of K_m and V_{max} were calculated as described in section 2. The value of V_{max} in every case was set relative to the activity observed with 10 mM L-carnitine in the absence of sulphonylureas. [A, B, C] indicate $P < 0.05$, 0.01, < 0.005, respectively, for effects of the sulphonylureas. [NS] indicates non-significant ($P = 0.34$).

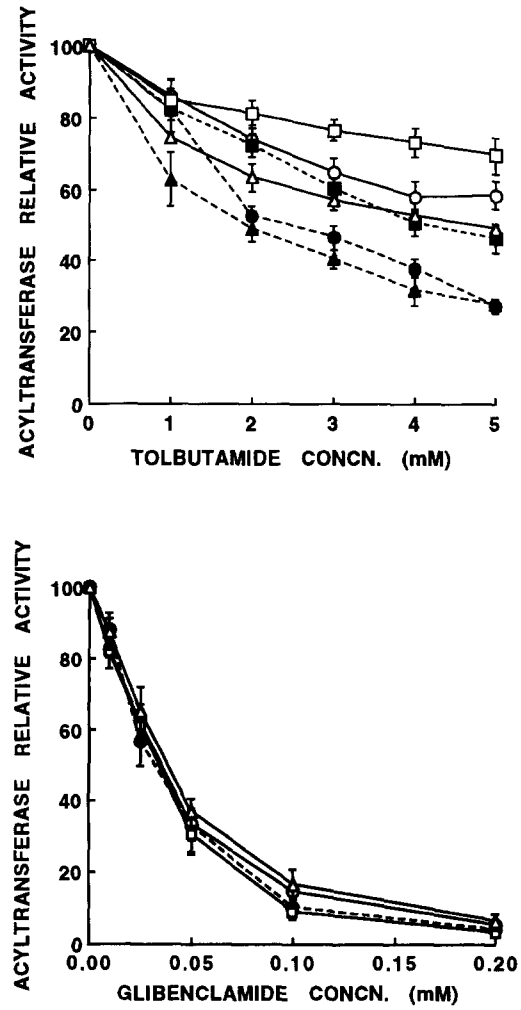

Fig. 1. Effects of tolbutamide and glibenclamide on microsomal malonyl-CoA-sensitive CAT activity. Values are means ± S.E.M. from 3 separate preparations in each case. ●, ○; malonyl-CoA-sensitive CAT in microsomal membrane residues depleted of malonyl-CoA-insensitive CAT by freezing/thawing. ■, □; malonyl-CoA-sensitive CAT after solubilization and partial purification by gel-filtration. ▲, △; partially-purified malonyl-CoA-sensitive CAT after reconstitution into L-α-lecithin liposomes. Closed symbols, [carnitine] = 0.1 mM; open symbols, [carnitine] = 2 mM.

whereas glibenclamide was 5 to 10 times less effective as an inhibitor of the malonyl-CoA-insensitive enzyme (it was not possible to test concentrations of glibenclamide in excess of 200 μM because of its limited solubility).

The effects of these two sulphonylurea drugs noted here have some similarities and some differences from their effects on the mitochondrial CATs shown by [10]. We found the concentration of tolbutamide that produced 50% inhibition of the malonyl-CoA-sensitive enzyme to be 70 to 150 times greater (depending on assay conditions) than the concentration of glibenclamide needed for an equivalent degree of inhibition. In broadly similar experiments Cook [10] noted that glibenclamide was 35 times more potent than tolbutamide in inhibiting the mitochondrial malonyl-CoA-sensitive CAT (it should be noted that the bovine albumin in the assays will bind both drugs and that glibenclamide binds more tightly than tolbutamide [16]). When carnitine concentration was varied the effect of gliben-

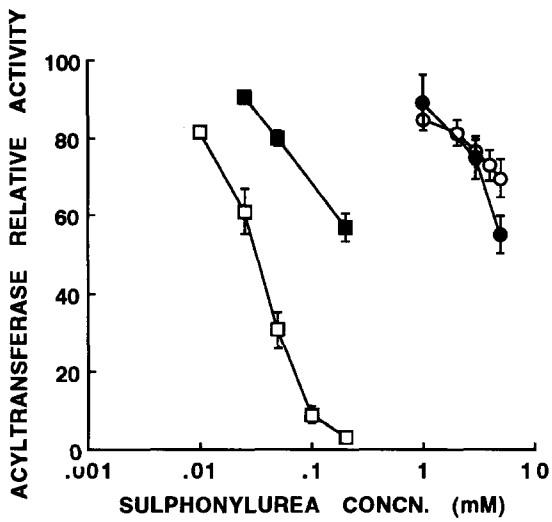

Fig. 2. Comparison of the effects of tolbutamide and glibenclamide on solubilized, partially purified preparations of microsomal malonyl-CoA-sensitive and malonyl-CoA-insensitive carnitine acyltransferase activities. Values are means ± S.E.M. from 3 separate preparations in each case. All CAT assays contained 2 mM L-carnitine. □, ■, with glibenclamide; ○, ●, with tolbutamide. Open symbols, malonyl-CoA-sensitive CAT; closed symbols, malonyl-CoA-insensitive CAT.

clamide on the microsomal malonyl-CoA-sensitive CAT was non-competitive but was uncompetitive with the mitochondrial malonyl- CoA-sensitive CAT [10]. The observation (Fig. 2) that the two microsomal CATs clearly differ from each other in their sensitivity to glibenclamide reinforces arguments presented elsewhere [5,6] that these are different enzymes.

The sulphonylurea drugs are widely used as adjuncts to dietary measures in the treatment of non-insulin-dependent diabetes by inhibiting B cell K_{ATP} channels following interaction with the recently cloned sulphonylurea receptor [17]. It may be speculated that CATs might have some similarity to the sulphonylurea receptor in the molecular motif(s) involved in the recognition of these drugs. Since the physiological role of the microsomal CATs is not yet known it is not possible to tell whether any of the various documented extrapancreatic effects of the sulphonylureas could result from interactions with microsomal CATs. We are however struck by the facts that 5 mM tolbutamide inhibited VLDL secretion by rat hepatocytes by 75% [8] and (at physiological carnitine concentrations) also inhibited the liver microsomal malonyl-CoA-sensitive CAT by 75% (Fig. 1).

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