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Gliclazide hydroxylation by rat liver microsomes

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1. The metabolism of gliclazide to hydroxygliclazide has been investigated in Sprague–Dawley rat liver microsomes.

2. The kinetics of hydroxygliclazide formation are consistent with Michaelis–Menten kinetics (mean (± SD, $n = 3$) apparent K_m and $V_{max} = 256 \pm 27\ \mu M$ and $1{\cdot}85 \pm 0{\cdot}10$ nmol/min/mg respectively).

3. Tolbutamide competitively inhibited hydroxygliclazide formation ($K_i = 840\ \mu M$) and gliclazide competitively inhibited hydroxytolbutamide formation ($K_i = 240\ \mu M$) with K_i similar to K_m. Therefore gliclazide and tolbutamide may be metabolized by the same enzyme in the rat. In nine livers the formation of hydroxygliclazide correlated with the formation of hydroxytolbutamide ($r_s = 0{\cdot}82$, $p < 0{\cdot}01$).

4. Diclofenac ($K_i = 64\ \mu M$), phenytoin ($K_i = 38\ \mu M$), mephenytoin ($K_i = 66\ \mu M$), glibenclamide ($K_i = 14\ \mu M$) and glipizide ($K_i = 189\ \mu M$) were fully competitive inhibitors of gliclazide hydroxylation. The rank order of K_i constants differed for gliclazide and tolbutamide suggesting that gliclazide and tolbutamide hydroxylases are not identical enzymes.

5. Quinine ($K_i = 0{\cdot}3\ \mu M$) and quinidine ($K_i = 4{\cdot}3\ \mu M$) were partially competitive inhibitors of hydroxygliclazide formation. Hydroxylation of gliclazide was related to the activity of CYP2D1 as assessed by dextrorphan production from dextromethorphan ($r_s = 0{\cdot}83$, $p = 0{\cdot}01$).

6. In the rat gliclazide is metabolized to hydroxygliclazide by at least two cytochrome P450 isoforms, including tolbutamide hydroxylase and 2D1, which have similar affinities for gliclazide.

Introduction

Gliclazide is a second generation sulphonylurea used in the treatment of type 2 diabetes. Gliclazide is eliminated principally via metabolism and eight metabolites have been identified in both rat and man (Campbell *et al.* 1980). The two major metabolites are hydroxygliclazide and carboxygliclazide (figure 1). The structurally related first generation sulphonylurea tolbutamide is also eliminated principally as hydroxy- and carboxy- metabolites (figure 1).

Since Scott and Poffenbarger (1979) suggested a possible genetic control of tolbutamide metabolism, the formation of hydroxytolbutamide has been extensively studied both *in vitro* and *in vivo* in rats (Back *et al.* 1984, Veronese *et al.* 1990, Bélanger *et al.* 1991) and man (Darby *et al.* 1972, Peart *et al.* 1987, Purba *et al.* 1987, Back *et al.* 1988, Miners *et al.* 1988). The oxidation of tolbutamide to hydroxytolbutamide is a P450-mediated reaction and in man is catalysed by the P450 isoform 2C9 (Veronese *et al.* 1991). The oxidation of a number of clinically important drugs including phenytoin (Doecke *et al.* 1991, Tassaneeyakul *et al.* 1992),

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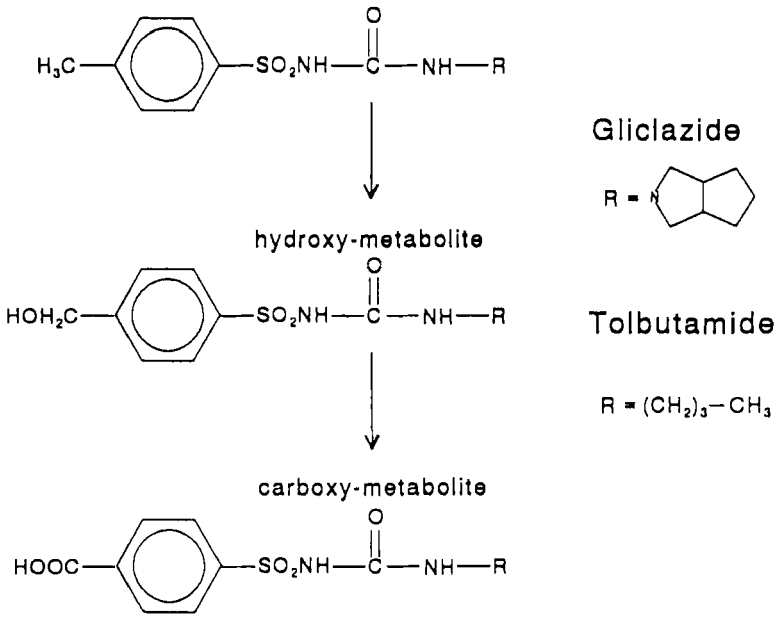

Figure 1. Chemical structures of gliclazide and tolbutamide and their pathways of oxidative metabolism.

S-warfarin (Rettie *et al.* 1992), diclofenac and other non-steroidal anti-inflammatory drugs (Leemann *et al.* 1993) is also mediated by this isoform. The P450 isoform responsible for the metabolism of tolbutamide to hydroxytolbutamide in the rat has not been identified. The metabolism of hydroxytolbutamide to carboxytolbutamide is catalysed by cytosolic alcoholic and aldehyde dehydrogenases in the rat (McDaniel *et al.* 1969).

Although oxidative biotransformation is a pivotal step in the elimination of gliclazide, no information on the nature and characteristics of the enzyme(s) catalysing the metabolism of gliclazide has been reported in the literature. In this initial investigation, the formation of hydroxygliclazide, the major pathway of gliclazide metabolism, has been characterized *in vitro* using rat liver microsomes. This reaction has been related to the formation of hydroxytolbutamide and the activity of the rat P450 isoform 2D1 (CYP2D1). The influence of classical inhibitors/substrates of human P450s and the related second-generation sulphonylureas, glipizide and glibenclamide, on the metabolism of both gliclazide and tolbutamide *in vitro* in rat liver microsomes has also been investigated.

Materials and methods

Chemicals and reagents

Glucose 6-phosphate (G6P) and glucose 6-phosphate dehydrogenase (G6PDH) were obtained from Boehringer Mannheim (Castle Hill, Australia). Chlorzoxazone, 3-(α-acetonylbenzyl)-4-hydroxycoumarin sodium (warfarin), dextromethorphan hydrobromide monohydrate, 5,5-diphenylhydantoin (phenytoin), glibenclamide, quinine, quinidine, salicylic acid, tolbutamide, troleandomycin (TAO) and β-$NADP^+$ (potassium salt) were purchased from the Sigma Chemical Co. (St Louis, MO, USA). Other drugs and drug metabolites were generously provided by the following sources:hydroxytolbutamide and carboxytolbutamide from Hoechst (Melbourne, Australia); hydroxygliclazide, carboxygliclazide and gliclazide from Servier Laboratories (Hawthorn, Australia); glipizide from Farmitalia Carlo Erba (Clayton, Australia); chlorpropamide from Pfizer (West Ryde, Australia); diclofenac sodium from Ciba-Geigy (Pendle Hill, Australia); mephenytoin from Sandoz (North Ryde, Australia); cimetidine hydrochloride from SmithKline Beecham (Dandenong, Australia), and dextrorphan tartrate from Roche

Products (Dee Why, Australia). All other reagents and solvents were of analytical grade and obtained from Ajax Chemicals (Auburn, Australia).

Microsome preparation

Livers were obtained from nine male Sprague–Dawley rats (460 ± 47 g) killed by cervical dislocation. Prior to removal, the livers were perfused via the portal vein with 20 ml cold normal saline. The livers were then placed immediately into liquid nitrogen and stored at −80°C until used. Microsomes were prepared from individual livers by differential centrifugation according to the method described by Meier *et al.* (1983). The microsomes were stored at −80°C in 500-μl aliquots in 0·05 M potassium phosphate buffer, pH = 7·4 containing 20% v/v glycerol. Cytochrome P450 content was measured spectrophotometrically according to Omura and Sato (1964) and protein concentration was quantified using the Pierce BCA Protein Assay (Rockford, IL, USA). This study was approved by the Animal Care and Ethics Committee Royal North Shore Hospital and University of Technology Sydney (Protocol No 9204-14 A).

Assay of gliclazide and tolbutamide hydroxylase activities

The substrates gliclazide or tolbutamide were added to 10-ml polypropylene tubes in amounts appropriate for the final incubation volume of 1 ml. After evaporation of the methanol present, microsomal protein (0·4 mg) and an appropriate volume of potassium phosphate buffer (0·1 M, pH = 7·4) were added and the tubes were preincubated for 4 min at 37°C. All reactions were started by the addition of the NADPH-generating system (1 mM $NADP^+$, 5 mM $MgCl_2$, 10 mM G6P, 2IU G6PD) and were carried out in air at 37°C in a shaking water bath for 20 min in a final volume of 1 ml. For all experiments the reaction was stopped by adding acid and rapid cooling on ice. For gliclazide incubations 200 μl 0·4 M H_3PO_4 was added and for tolbutamide 100 μl 2 M HCl was used. Initial experiments were performed by varying the protein content (0·1–1·0 mg) and incubation times (5–60 min) to assess the linearity of these parameters. In experiments to determine the kinetic parameters for hydroxygliclazide or hydroxytolbutamide formation, twenty gliclazide or tolbutamide concentrations (2–2000 μM) were studied.

To examine the effects of cytochrome P450 inhibitors on the formation of hydroxygliclazide or hydroxytolbutamide, substrate concentrations of 240 and 400 μM similar to the respective K_m's, were selected. Compounds screened for possible inhibitory effects are listed in table 2 and two inhibitor concentrations, generally 50 and 500 μM were used. The per cent of activity remaining was calculated relative to controls run on the same day without inhibitors (100%). Inhibitors were added in potassium phosphate buffer 0·1 M, pH = 7·4 or methanol and appropriate controls were always performed. Compounds reducing hydroxy-metabolite production by < 20% at 500 μM were designated non-inhibitors. For a number of inhibitors a full kinetic study was performed using the same batch of microsomes. The constant of inhibition (K_i) and the nature of inhibition were characterized. Three substrate concentrations (0·5 × K_m, K_m and 2 × K_m) were used to determine the K_i's.

Hydroxytolbutamide was assayed according to Miners *et al.* (1988) with some minor modifications. This method was also modified as follows to assay hydroxygliclazide. Briefly, gliclazide (or tolbutamide) was partly removed from the microsomal incubate by extraction with 8 ml hexane : $CHCl_3$: isobutyl alcohol (100 : 25 : 0·5; v/v/v). Of the internal standard, chlorpropamide 100 μl (2 mg/l for gliclazide; 10 mg/l for tolbutamide) was added and the aqueous phase re-extracted with 3 ml diethylether. The upper organic layer was transferred to a clean polypropylene tube and evaporated to dryness at 60°C under a gentle flow of nitrogen. The residue was reconstituted in 100 μl mobile phase and an aliquot (40 μl) injected on column. The mobile phase consisted of acetonitrile : 5 mM acetate buffer pH 4·3 (28 : 72 for gliclazide; 25 : 75 for tolbutamide) and was pumped at a flow rate of 1·5 ml/min using a 510 Solvent Delivery Module (Waters Associates, Milford, USA). The UV absorbance was monitored at 230 nm with a Lambda-Max Model 481 detector (Waters Associates). For the gliclazide assay (figure 2) the retention times were 1·8 min for carboxygliclazide, 3·2 min for hydroxygliclazide, 5·9 min for chlorpropamide, and 17 min for gliclazide. For the tolbutamide assay, the retention times were 1·2 min for carboxytolbutamide, 2·7 min for hydroxytolbutamide, 8 min for chlorpropamide, and 15 min for tolbutamide. Standard curves were prepared by adding varying amounts of hydroxygliclazide (0·1–12 nmol) or hydroxytolbutamide (0·1–18 nmol) to potassium phosphate buffer 0·1 M pH 7·4 (final volume 1 ml). Unknown concentrations were determined from the relationship between analyte concentration and peak area ratio of the standards as determined by least-square linear regression analysis.

Quality control specimens to monitor the performance of both the hplc assay and the microsomal incubation technique were measured with each experimental run. The within- and between-day reproducibilities of the hplc technique were determined on six occasions at three different concentrations (0·25, 3, 9 nmol) of hydroxygliclazide or hydroxytolbutamide. The coefficients of variation were < 6%. The within-day coefficients of variation of the microsomal incubation technique were determined by measuring hydroxygliclazide formation in six samples at three different gliclazide concentrations (5, 250, 1500 μM). At each concentration the variation measured was < 5%. To assess the reliability of the results from each experimental run, microsomes were prepared from the combined livers of six rats. The formation of hydroxygliclazide or hydroxytolbutamide was measured at three substrate concentrations on each study day using these pooled microsomes. The results of experiments were rejected if the pooled microsome results varied by > 25% from the mean.

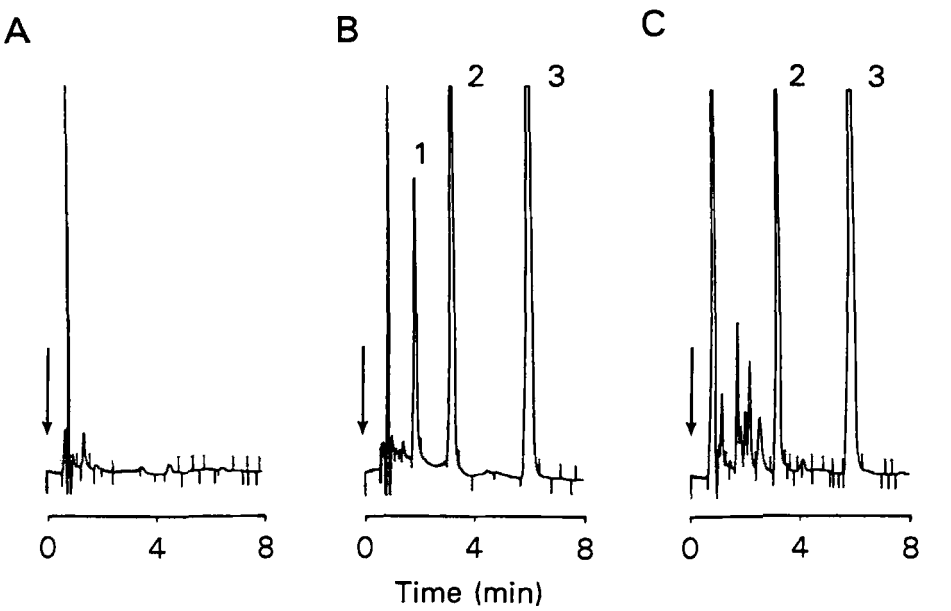

Figure 2. Representative chromatograms: (A) microsomal incubation experiment performed without gliclazide and no internal standard has been added; (B) microsomal incubation experiment performed without substrate. Carboxy-gliclazide 3 μM, hydroxygliclazide 3 μM and internal standard 200 μg/l have been added; and (C) microsomal incubation performed with gliclazide 240 μM. The hydroxygliclazide concentration is 3·2 μM. 1, 2 and 3 annotate the carboxygliclazide, hydroxygliclazide, and internal standard peaks respectively.

Assay of CYP2D1 activity

To monitor CYP2D1 activity in the rat liver microsomes, the formation of dextrorphan from dextromethorphan was characterized (Kerry *et al.* 1993, Hoskins 1994). For these experiments a final incubation volume of 200 μl was used. The NADPH-regenerating system (1 mM $NADP^+$, 5 mM $MgCl_2$, 10 mM G6P, 0·4 IU G6PD) and 0·1 M potassium phosphate buffer (pH = 7·4) were preincubated with dextromethorphan (0·6 μM) for 5 min at 37°C. The reaction was initiated by adding 5 μg microsomal protein and stopped at 5 min by adding 10 μl $HClO_4$ 17·5%. The incubates were vortexed and centrifuged for 8 min at 9500*g*. An aliquot (130 μl) of the supernatant was injected on to the hplc column to measure the formation of dextrorphan.

Data treatment

All experiments were performed in duplicate and the mean is reported. Initial estimates of K_m (Michaelis–Menten constant) and V_{max} (maximum rate of formation) were obtained by least-squares linear regression analysis of the data plotted as rate of formation against concentration (Hofstee 1952). These values were used as initial estimates to fit the data to the Michaelis–Menten equation using MK MODEL (Holford 1994). The method of Dixon was used to calculate inhibition constants (K_i) (Dixon 1953). As this method cannot distinguish competitive from mixed-type inhibition (Dixon and Webb 1979), the methods of Cornish-Bowden (Cornish-Bowden 1974) and Lineweaver–Burk (Dixon and Webb 1979) were used to evaluate the type of inhibition. If the curves of the Cornish-Bowden plot at different substrate concentrations were parallel, the inhibition was considered competitive. In addition the inhibition was considered competitive if the graph of the slopes of the Lineweaver–Burk plots, plotted against the inhibitor concentration, was linear. If this graph was hyperbolic the inhibition was considered partially competitive. In the presence of a partially competitive inhibitor, K_i was determined graphically from the relationship between the reciprocal of the slopes of the Lineweaver–Burk plots and the reciprocal of the inhibitor concentration (Dixon and Webb 1979).

Spearman rank correlation coefficients (r_s) were calculated to assess any relationship between the rate of formation of hydroxygliclazide and hydroxytolbutamide or dextrorphan production.

Results

A sensitive and reproducible hplc method has been developed to measure hydroxygliclazide and carboxygliclazide concentrations in microsomal incubates. Figure 2 shows representative chromatograms for the gliclazide metabolite assay. The mean recovery of hydroxygliclazide was 60%. Hydroxygliclazide was formed in all microsomal incubates in the presence of the NADPH-generating system; however, in its absence, no hydroxygliclazide was observed (data not shown). Under aerobic conditions and in the presence of the NADPH-generating system carboxygliclazide could not be detected in any microsomal incubate. Gliclazide 4-hydroxylation increased linearly with time up to 40 min and with protein content up to 0·6 mg (data not shown). The standard conditions selected, a 20-min incubation time and 0·4 mg protein, were therefore within the appropriate linear ranges.

The formation of both hydroxygliclazide and hydroxytolbutamide was consistent with Michaelis–Menten kinetics. Figure 3 shows the relationship between substrate concentration and rate of formation of hydroxygliclazide and hydroxytolbutamide. The K_m and V_{max} calculated are given in table 1.

Gliclazide and tolbutamide were mutually competitive inhibitors of microsomal oxidation. Figure 4 shows the Dixon plot obtained for the inhibition of hydroxygliclazide formation by tolbutamide, and the Cornish-Bowden plot representing the inhibition of hydroxytolbutamide formation by gliclazide. For both drugs acting as either substrates or inhibitors the lines of the Cornish-Bowden plot were parallel indicating that the inhibition was fully competitive. Gliclazide inhibited hydroxytolbutamide formation with a $K_i = 240\,\mu M$ and tolbutamide inhibited hydroxygliclazide formation with a $K_i = 840\,\mu M$. These K_i's were similar to the respective K_m's (table 1).

The influence of cytochrome P450 inhibitors on the formation of both hydroxygliclazide and hydroxytolbutamide is shown in table 2. Of the compounds screened, only salicylic acid was a non-inhibitor of both hydroxygliclazide and hydroxytolbutamide formation. The general P450 inhibitor, cimetidine, significantly inhibited the metabolism of gliclazide. All the other compounds tested inhibited hydroxygliclazide formation by $>20\%$ at their highest concentration. Apparent K_i's for some of these compounds, calculated from full kinetic studies, are given in table 3. The type of inhibition as judged from Cornish-Bowden plots and the interpretation of the graphical retreatment of Lineweaver–Burk plots is also given in table 3. The compounds differed in their potency to inhibit either hydroxygliclazide or hydroxytolbutamide production. For example, mephenytoin was a more potent inhibitor of hydroxygliclazide ($K_i = 70\,\mu M$) than hydroxytolbutamide ($K_i = 190\,\mu M$). Conversely, cimetidine (table 2) and diclofenac (table 3) were more potent inhibitors of tolbutamide than of gliclazide hydroxylase. The formation of hydroxygliclazide and hydroxytolbutamide was inhibited to a similar degree by the sulphonylureas glibenclamide and glipizide.

The relationships observed between the formation of hydroxygliclazide ($60\,\mu M$ gliclazide), hydroxytolbutamide ($100\,\mu M$ tolbutamide), and dextrorphan ($0{\cdot}6\,\mu M$ dextromethorphan) in different livers are shown in figure 5. Hydroxygliclazide formation was related to both hydroxytolbutamide formation ($r_s = 0{\cdot}82$, $p < 0{\cdot}01$, $n = 9$) and dextrorphan formation ($r_s = 0{\cdot}83$, $p < 0{\cdot}05$, $n = 8$). However, the dextromethorphan *O*-demethylase and tolbutamide hydroxylase activities were not significantly correlated ($r_s = 0{\cdot}38$, $p < 0{\cdot}05$, $n = 8$).

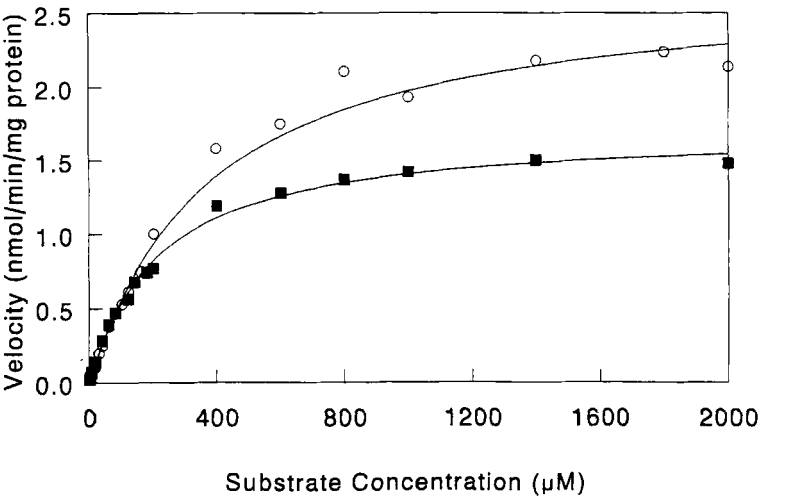

Figure 3. Relationship between substrate (S) concentration and velocity (V) of rate of formation of hydroxygliclazide (■) and hydroxytolbutamide (○). Means of duplicate determinations using microsomes from a single rat liver are shown.

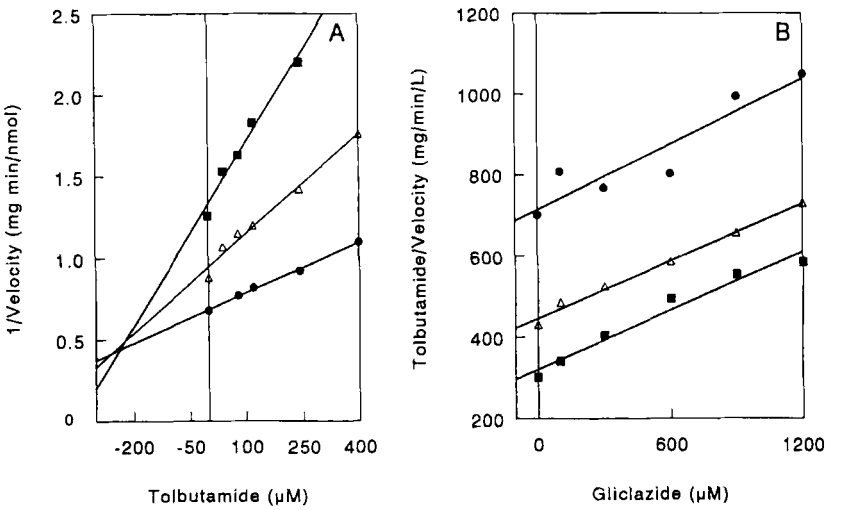

Figure 4. Reciprocal inhibition of gliclazide and tolbutamide hydroxylation in microsomes from a single rat liver. Each experiment was performed in duplicate and the means are shown. (A) Dixon plot for the inhibition of hydroxygliclazide formation by tolbutamide. Concentrations of gliclazide were 120 (■), 240 (△), and 480 μM (●). (B) Cornish-Bowden plot for the inhibition of hydroxytolbutamide formation by gliclazide. Concentrations of tolbutamide were 200 (■), 400 (△), and 800 μM (●).

Discussion

Despite the widespread use of gliclazide in the treatment of type 2 diabetes and the fact that it is eliminated principally by metabolism, information identifying the enzyme(s) that metabolize gliclazide or the kinetics of metabolism is not available in the literature. As the factors influencing the expression and activity of the cytochrome P450 isoforms and their substrate specificities are becoming increasingly understood, identifying the enzyme(s) catalysing gliclazide metabolism will contribute substantially to our understanding of factors influencing gliclazide

Table 1. Michaelis–Menten parameters (mean ± SD) for hydroxygliclazide and hydroxytolbutamide formation by rat liver microsomes ($n = 3$).

	Apparent K_m (μM)	V_{max} (nmol/min/mg)
Gliclazide	256 ± 27	1·85 ± 0·10
Tolbutamide	441 ± 38	2·36 ± 0·22

Table 2. Inhibitory effects of various compounds on hydroxygliclazide and hydroxytolbutamide formation. Values represent activity as percent of control activity and are the mean of duplicate determinations (control = 100%).

	Gliclazide (240 μM)		Tolbutamide (400 μM)	
Compound	50 μM	500 μM	50 μM	500 μM
Salicylic acid	93	80	107	107
Cimetidine	31	7	84	50
Warfarin	59	21	80	31
Diclofenac	58	19	20	5
Phenytoin	47	30*	100	85*
Mephenytoin	71	22	96	57
Quinine	20	2	61	20
Quinidine	25	7	97	53
TAO	89	66	101	92
Chlorzoxazone	73	nd	97	nd
Glipizide	90	59*	89	73*
Glibenclamide	32	2	56	13

nd, Not determined.

* The highest concentrations used for glipizide and phenytoin were 300 and 150 μM respectively, because of their limited solubilities.

disposition. Metabolism of gliclazide to hydroxygliclazide is the initial step in the metabolism of gliclazide in both rat and man (Oida *et al.* 1985). In the current study a simple hplc method with adequate sensitivity to measure hydroxygliclazide concentrations in microsomal incubates has been developed. This technique has enabled the kinetics of the formation of hydroxygliclazide by rat liver microsomes to be characterized and related to the metabolism of tolbutamide to hydroxytolbutamide.

The formation of hydroxygliclazide in the microsomal incubates was dependent on the presence of an NADPH-generating system. Carboxygliclazide was not observed in any microsomal incubate, indicating that the oxidation of hydroxygliclazide itself is not catalysed by microsomal enzymes. Oida *et al.* (1985) suggested that cytosolic alcohol and aldehyde dehydrogenases catalyse this biotransformation as these enzymes catalyse the formation of carboxytolbutamide from hydroxytolbutamide (McDaniel *et al.* 1969), an analogous reaction.

In all three livers studied, hydroxygliclazide and hydroxytolbutamide formation were consistent with Michaelis–Menten kinetics. K_m's for gliclazide were lower than for tolbutamide, indicating that the hydroxylating enzyme has a higher affinity for gliclazide (table 1). The K_m and V_{max}'s for tolbutamide observed in Sprague–Dawley rat liver microsomes in this study were similar to the values reported in the literature for the Wistar rat ($K_m = 552\ \mu$M; $V_{max} = 1{\cdot}18$ nmol/min/mg) (Veronese *et al.* 1990).

Table 3. Inhibition constants (K_i) of various compounds for gliclazide and tolbutamide hydroxylation by rat liver microsomes. Type of inhibition as determined from Cornish-Bowden or graphical retreatment of Lineweaver–Burk plots is also given. Each experiment was performed in duplicate and the means are reported.

Compound	Hydroxygliclazide formation K_i (μM)	Type of inhibition	Hydroxytolbutamide formation K_i (μM)	Type of inhibition
Diclofenac	64	C	7	C
Phenytoin	38	C	nd	nd
Mephenytoin	66	C	189	C
Quinine	0·3	PC	9·1	PC
Quinidine	4·3	PC	130	PC
Glibenclamide	14	C	25	C
Glipizide	189	C	197	C

nd, Not determined.
C, fully competitive; and PC, partially competitive.

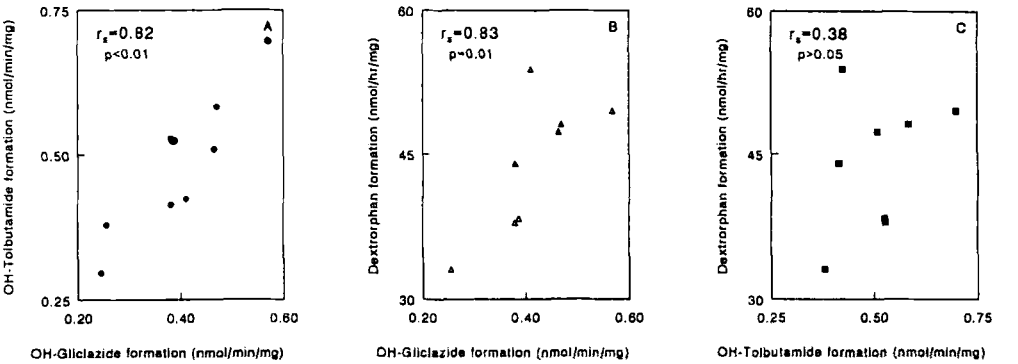

Figure 5. Relationship between the activities of three enzymes* in rat liver: (A) hydroxytolbutamide and hydroxygliclazide formation ($n = 9$); (B) hydroxygliclazide and dextrorphan formation ($n = 8$); and (C) hydroxytolbutamide and dextrorphan formation ($n = 8$). * Hydroxytolbutamide: OH-tolbutamide; hydroxygliclazide: OH-gliclazide.

The kinetic studies could not distinguish the interaction of gliclazide with more than one enzyme which all have similar affinities for gliclazide.

Gliclazide and tolbutamide inhibited each other's hydroxylation in a competitive manner (figure 4) with K_i's similar to their respective K_m's (table 1). Whereas the lack of inhibition of one substrate by another is reasonable evidence that different enzymes are involved in the metabolism of the two compounds, competitive inhibition does not necessarily indicate metabolism by the same isoenzyme (Dixon and Webb 1979). However in nine rat livers hydroxygliclazide and hydroxytolbutamide formation was significantly correlated ($r_s = 0{\cdot}82$, $p < 0{\cdot}01$). These observations suggest that both reactions are mediated by at least one common P450 isoform in the rat.

Several compounds known to be inhibitors of different human P450 isoforms and two sulphonylurea drugs, glipizide and glibenclamide, were screened for their effects on hydroxygliclazide formation in rat liver microsomes. Species' differences in the activity and substrate specificity of the enzymes of the cytochrome P450 family are widely known. Rat cytochrome P450 isoforms do not appropriately reflect the substrate specificity or activity of human tolbutamide hydroxylase, CYP2C9

(Veronese *et al.* 1990). Therefore in this initial study of gliclazide metabolism in the rat our results could not be related to those of previous *in vitro* studies of tolbutamide metabolism in man or to individual human cytochrome P450 isoforms.

The hydroxylation of both gliclazide and tolbutamide was inhibited by cimetidine, a general P450 inhibitor, and in a competitive manner by substrates of the human enzymes CYP2C9 (phenytoin and diclofenac) and CYP2C19 (mephenytoin). A different rank order of potency of inhibition was observed for tolbutamide and gliclazide (table 2). If the same rat enzyme were metabolizing both gliclazide and tolbutamide we would expect a similar rank order of potency for the range of inhibitors investigated.

Quinine and quinidine were partially competitive inhibitors of gliclazide hydroxylation (table 3). The inhibition constant for quinine ($K_i = 0{\cdot}3\ \mu M$) was the lowest of all compounds tested. Quinine was 14 times more potent than quinidine in inhibiting hydroxygliclazide ($K_i = 4{\cdot}3\ \mu M$) and hydroxytolbutamide ($K_i = 9{\cdot}1\ \mu M$). Stereoselective inhibition by quinine and quinidine has been described for compounds whose metabolism is catalysed by CYP4502D1 (Kobayashi *et al.* 1989). Interestingly the formation of hydroxygliclazide was also related to the activity of CYP4502D1 as assessed by the *O*-demethylation of dextromethorphan ($r_s = 0{\cdot}83$) but it correlated poorly ($r_s = 0{\cdot}38$) with hydroxytolbutamide formation. These observations also suggest that gliclazide and tolbutamide are not solely metabolized by the same cytochrome P450 isoform.

Finally, glipizide and glibenclamide, two sulphonylurea drugs structurally related to tolbutamide and gliclazide, were competitive inhibitors of both tolbutamide and gliclazide hydroxylation with comparable inhibition constants. The glibenclamide inhibition constants were substantially lower than those for glipizide, indicating that glibenclamide has a higher affinity for the metabolizing enzyme(s). Glibenclamide and glipizide are eliminated principally by oxidative metabolism in man and, like gliclazide, their metabolism has not been characterized *in vitro*. Our results suggest that these drugs may also be metabolized by the enzyme(s) catalysing the biotransformation of tolbutamide and gliclazide.

As hydroxygliclazide formation was consistent with Michaelis–Menten kinetics, we propose that in the rat at least two cytochrome P450 isoforms, with similar affinities for gliclazide, catalyse its formation. Our data indicate that both rat 'tolbutamide hydroxylase' and CYP2D1 catalyse the hydroxylation of gliclazide in the rat. Further studies to examine the role of CYP2C9 and CYP2D6 in the metabolism of gliclazide in man are warranted.

Acknowledgements

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