Decreased Glibenclamide Uptake in Hepatocytes of Hepatocyte Nuclear Factor-1α–Deficient Mice

A Mechanism for Hypersensitivity to Sulfonylurea Therapy in Patients With Maturity-Onset Diabetes of the Young, Type 3 (MODY3)

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Diabetes in subjects with hepatocyte nuclear factor (HNF)-1α gene mutations (maturity-onset diabetes of the young [MODY]-3) is characterized by impaired insulin secretion. Surprisingly, MODY3 patients exhibit hypersensitivity to the hypoglycemic actions of sulfonylurea therapy. To study the pharmacogenetic mechanism(s), we have investigated glibenclamide-induced insulin secretion, glibenclamide clearance from the blood, and glibenclamide metabolism in wild-type and Hnf-1α–deficient mice. We show that despite a profound defect in glucose-stimulated insulin secretion, diabetic Hnf-1α–/– mice have a robust glibenclamide-induced insulin secretory response. We demonstrate that the half-life (t1/2) of glibenclamide in the blood is increased in Hnf-1α–/– mice compared with wild-type littermates (3.9 ± 1.3 vs. 1.5 ± 1.8 min, P ≤ 0.05). The clearance of glibenclamide from the blood during the first hours after intravenous administration was reduced approximately fourfold in Hnf-1α–/– mice compared with Hnf-1α+/+ littermates. Glibenclamide uptake into hepatocytes was dramatically decreased in vivo and in vitro. To study the metabolism of glibenclamide in Hnf-1α–/– animals, we analyzed liver extracts from [3H]glibenclamide-injected animals by reverse-phase chromatography. We found that the ratio of the concentrations of glibenclamide and its metabolites was moderately increased in livers of Hnf-1α–/– mice, suggesting that hepatic glibenclamide metabolism was not impaired in animals with Hnf-1α deficiency. Our data demonstrate that high serum glibenclamide concentrations and an increased t1/2 of glibenclamide in the blood of Hnf-1α–/– mice are caused by a defect in hepatic uptake of glibenclamide. This suggests that hypersensitivity to sulfonylureas in MODY3 patients may be due to impaired hepatic clearance and elevated plasma concentrations of the drug. Diabetes 51 (Suppl. 3):S343–S348, 2002

Mutations in the hepatocyte nuclear factor (HNF)-1α gene cause the most common form of maturity-onset diabetes of the young (MODY), type 3 (1). Clinical studies in humans indicate that β-cell dysfunction in MODY3 patients leads to a defect in glucose-stimulated insulin secretion that is responsible for chronic hyperglycemia (2). Mice lacking the Hnf-1α gene (3,4) also have a dramatic reduction in insulin secretory response to glucose. Insulin secretion from islets of Hnf-1α–/– mice is blunted in response to glucose and other glycolytic stimuli but normal in response to non-nutrient secretagogues such as potassium (5). Furthermore, the expression of GLUT2 and the glycolytic enzymes glucokinase and liver pyruvate kinase are reduced in pancreatic islets of Hnf-1α–/– mice compared with wild-type mice (6). Therefore, a defect in the β-cell glycolytic pathway contributes to diabetes in Hnf-1α–/– mice and MODY3 patients.

Despite the profound reduction of insulin responses to glucose stimulation, MODY3 patients have been shown to be hypersensitive to the actions of sulfonylurea drugs with symptoms of hypoglycemia developing 60–90 min after as little as 0.5 mg glibenclamide orally (7–9). Hypersensitivity to sulfonylureas can occur in both early and late stages of the disease as well as in glucose-tolerant or diabetic subjects with HNF-1α mutations. Furthermore, increased sensitivity to glibenclamide has been shown to cosegregate with mutation hot spot P291fsinsC in one Norwegian family, suggesting that a pharmacogenetic mechanism may be responsible for the altered pharmacological response to glibenclamide treatment (8).

Sulfonylureas cause hypoglycemia by stimulating insulin release from β-cells. The effects of sulfonylureas are initiated by drug binding to the ATP-sensitive K+ (K_{ATP}) channel, a complex that consists of two subunits: the sulfonylurea receptor (SUR1) and an inward rectifier channel protein (Kir6.2), located in the plasma membrane of β-cells (10,11). Interaction of sulfonylurea and receptor proteins with the sulfonylurea receptor (SUR1) and an inward rectifier channel protein (Kir6.2), located in the plasma membrane of β-cells (10,11). Interaction of sulfonylurea and receptor...
induces membrane depolarization, activation of voltage-gated Ca\(^{2+}\) channel, and degranulation of insulin-containing vesicles (12). We have recently shown that the gene expression of Sur1 and Kir6.2 are normal in islets of Hnf-1\(^{-/-}\) mice (6). Thus, although \(b\)-cell K\(_\text{ATP}\) channels play a critical role in the regulation of glucose- and sulfonylurea-induced insulin secretion, their expression is not regulated by Hnf-1\(\alpha\) and is unlikely to be directly involved in the hypersensitivity to sulfonylureas.

HNF-1\(\alpha\) is expressed in pancreatic islets, liver, kidney, and intestine. Liver and kidney play a significant role in the detoxification of drugs. Therefore, HNF-1\(\alpha\) gene mutations could lead to liver or kidney dysfunction altering the pharmacokinetics of sulfonylureas in MODY3 patients. We have postulated that the unusual sensitivity to the hypoglycemic effect of sulfonylureas in these patients could be due to an impairment of drug elimination/metabolism. To test our hypothesis, we have used a murine model of MODY3, the Hnf-1\(\alpha\)-deficient (Hnf-1\(^{-/-}\)) mice, and glibenclamide (1-4-[2-(5-chloro-2-methoxybenzamido)ethyl]-benzenesulfonyl]-3-cyclohexylurea), a second-generation sulfonylurea compound. Glibenclamide is metabolized in the liver, and its metabolites are predominantly excreted in bile (13). Glibenclamide is a weak organic acid with a pKa (log of dissociation constant of acid) of 6.8 that dissociates to an organic anion at pH 7.4 (14). Hepatic uptake of glibenclamide has been studied in rat hepatocytes and shown to be transported by facilitated diffusion through a sodium- and energy-independent carrier mechanism (15,16).

In the present study, we examined the role of Hnf-1\(\alpha\) in the metabolism of glibenclamide. Our results indicate that loss of function of Hnf-1\(\alpha\) has a profound effect on glibenclamide uptake by hepatocytes and leads to decreased clearance from the plasma. Elevated glibenclamide levels may therefore be responsible for the hypersensitivity of sulfonylureas in subjects with HNF-1\(\alpha\) mutations.

**DECREASED GLIBENCLAMIDE UPTAKE IN HNF-1\(^{-/-}\) HEPATOCYTES**

**Preparation of primary hepatocytes.** Hepatocytes were prepared from 5- to 7-week-old Hnf-1\(\alpha\)-/- mice and their wild-type littermates according to the collagenase perfusion method of Ferre et al. (17) with the following modifications: 1) the livers were excised, transferred to Dulbecco’s modified Eagle’s medium (DMEM), and cut into small pieces. The resulting cell suspension was passed through nylon mesh filters, centrifuged at 50g, and resuspended in DMEM, supplemented with 10% fetal bovine serum to inhibit residual collagenase activity. The hepatocytes were plated onto gelatin-coated plates at a density of 10\(^6\) cells/cm\(^2\); cultured in DMEM, supplemented with 10% fetal bovine serum, pyruvate (110 mg/ml), t-glutamine (2 mM), penicillin, and streptomycin; and incubated at 37°C in 5% CO\(_2\) atmosphere. The viability of the cells was tested at 0.4% trypan blue and was 80--90%. Uptake experiments were performed within 24 h after the preparation of hepatocytes.

**Extraction and chromatographic separation.** Extraction and chromatographic separation were performed according to Ryberg et al. (18) using a modified protocol. Briefly, for extraction, 500 mg liver tissue was homogenized in 2 ml PBS, and the cytosolic fraction was prepared by centrifugation at 14,000g for 10 min at 4°C. Serum was diluted with five volumes PBS before extraction. Then, 10% (vol/vol) of 2 mol/l HCl was added to cytosolic fraction or serum, and extraction was performed with six volumes n-hexane-dichloromethane (1:1, vol/vol) for 10 min at room temperature. The organic phase was dried to a conical and evaporated to dryness at 37°C under a gentle stream of air, and the residue was dissolved in 50 ml methanol. Recoveries from liver and gut were 85% and from serum were 100%. For chromatographic separation of glibenclamide and its metabolites, a BioCAD Perfusion Workstation (PerSeptive Biosystems) with a reverse-phase C\(_18\) column (5 \(\mu\)m, 4.6 mm \(\times\) 250 mm, Vydac) was used. The system was run at room temperature, and the mobile phase contained acetonitrile-PBS (26:74, vol/vol). The flow rate was 1 ml/min with electrolytes ranging from 11 to 17 MPa. Before separation, the system was equilibrated with a mobile phase for 1 h. A total of 20 \(\mu\)l of the sample was injected, and 96 samples (0.2 ml) were collected starting at the time of injection. Radioactivity in all samples was measured by liquid scintillation counting. The recovery of radioactivity was 100%.

**Biochemical measurements.** Protein concentrations were quantified by the bicinchoninic acid protein assay (Sigma) according to the manufacturer’s instructions and using bovine serum albumin as a standard. Insulin concentrations were determined by a double-antibody radioimmunoassay (Linco, St. Louis, MO) using rat insulin as standard. 

**Statistical analysis.** Results are expressed as means \(\pm\) SE. Statistical analysis was performed by a two-tailed Student’s t test for unpaired data. Differences were considered to be significant at \(P<0.05\).

**RESULTS**

Glibenclamide-induced insulin secretion in mutant Hnf-1\(^{-/-}\) mice. To assess sulfonylurea-induced insulin secretion in vivo, we administered glibenclamide (1 mg/kg body wt) intravenously and compared insulin secretion between wild-type mice, Hnf-1\(^{-/-}\) mice, and their Hnf-1\(^{-/-}\) littermates. After glibenclamide injection, Hnf-1\(^{-/-}\) mice exhibited insulin levels at 2, 5, 10, 30, and 60 min that were indistinguishable from those of wild-type animals. Furthermore, blood glucose levels did not differ in Hnf-1\(^{-/-}\) and Hnf-1\(^{-/-}\) animals after intravenous glibenclamide administration. We then compared serum insulin levels after a glibenclamide challenge of Hnf-1\(^{-/-}\) and Hnf-1\(^{-/-}\) animals. Fasting insulin levels from blood samples collected at 15 and 0 min were comparable between the two groups of mice (0.27 \pm 0.06 and 0.36 \pm 0.11 ng/ml in Hnf-1\(^{-/-}\) vs. 0.27 \pm 0.03 and 0.24 \pm 0.04 ng/ml in Hnf-1\(^{-/-}\) animals). Insulin levels at 2 min after glibenclamide were not decreased in Hnf-1\(^{-/-}\) mice (2.48 \pm 0.38 ng/ml) compared with Hnf-1\(^{-/-}\) mice (2.36 \pm 0.32 ng/ml). As shown in Fig. 1, a similar pattern of insulin response was also observed at later time points,
disappearance of [3H]glibenclamide from the plasma was observed; glibenclamide levels decreased to only 50% after 2 min, and 96% after 5 min in mice. Glibenclamide was rapidly cleared (91% after 2 min and 96% after 5 min) in Hnf-1α−/− mice after a bolus intravenous injection. The rate of disappearance of [3H]glibenclamide from the plasma was markedly reduced in Hnf-1α−/− mice compared with Hnf-1α+/+ mice (Fig. 2). The ratio of the area under the curve of Hnf-1α−/− versus Hnf-1α+/+ was 4.2. Glibenclamide plasma concentrations remained significantly elevated for 2 h in Hnf-1α−/− mice compared with Hnf-1α+/+ mice. Glibenclamide was rapidly cleared (91% after 2 min and 96% after 5 min) in Hnf-1α+/+ mice after intravenous administration of [3H]glibenclamide. In contrast, plasma glibenclamide levels decreased to only 50% after 2 min and to 16% after 1 h in Hnf-1α−/− animals. The half-life of glibenclamide in Hnf-1α−/− and Hnf-1α+/+ mice was 30 s and 2 min, respectively. These results demonstrate that glibenclamide clearance from the blood is markedly reduced in Hnf-1α−/− mice. Because the liver is the major site for drug metabolism of glibenclamide, we hypothesized that the reduced clearance of glibenclamide was due to a decrease in hepatic uptake or impaired metabolism of the sulfonylurea.

Glibenclamide uptake is decreased in Hnf-1α−/− hepatocytes. Several extrinsic serum factors in Hnf-1α−/− mice could in theory be responsible for the decreased uptake of glibenclamide in mutant Hnf-1α−/− animals. For instance, Hnf-1α−/− mice have elevated bile acid serum levels that may inhibit glibenclamide uptake into hepatocytes in vivo (20,21). To rule out the potential involvement of inhibitors of glibenclamide transport, such as cholate, that may interact with glibenclamide clearance in vivo, we analyzed the uptake of [3H]glibenclamide into cultured primary hepatocytes that were isolated from Hnf-1α−/− and Hnf-1α+/+ littermate mice. The time course for the net uptake of [3H]glibenclamide into cultured hepatocytes is shown in Fig. 3A. Glibenclamide uptake into primary hepatocytes from Hnf-1α−/− mice was markedly lower than that of the hepatocytes derived from Hnf-1α+/+ livers. Kinetics parameters of glibenclamide uptake into cultured hepatocytes were determined using a Lineweaver-Burk plot analysis (data not shown). The calculated Vmax was 2.4-fold higher in Hnf-1α+/+ hepatocytes than in Hnf-1α−/− hepatocytes (301 vs. 128 pmol · min⁻¹ · mg⁻¹ protein, respectively). These results demonstrate that differences in glibenclamide up-

**FIG. 1.** Sulfonylurea-induced insulin response in vivo. Glibenclamide (1 g/kg body wt) was injected intravenously into 4- to 6-week-old wild-type (Hnf-1α+/+) and mutant (Hnf-1α−/−) mice that were fasted for 10–14 h. Blood samples were collected at the indicated time intervals by retro-orbital bleeds for measurements of insulin. Values are means ± SE (n = 4–6). No significant differences were observed between Hnf-1α+/+ and Hnf-1α−/− mice.

**FIG. 2.** Glibenclamide clearance is decreased in Hnf-1α−/− deficient mice. ([3H]glibenclamide (0.2 μCi/g; glibenclamide: 1 g/kg body wt) was injected into the tail vein. Blood samples were taken by retro-orbital bleeds at the indicated time intervals, and radioactivity was measured by liquid scintillation counting and expressed in nanomoles of glibenclamide per milliliter of plasma. Each value represents mean ± SE of six mice. **P < 0.01.

**FIG. 3.** Glibenclamide hepatic uptake is decreased in Hnf-1α−/− deficient mice. The time course of glibenclamide uptake in cultured primary hepatocytes from wild-type (Hnf-1α+/+) and mutant (Hnf-1α−/−) mice is shown. The final glibenclamide concentration in the medium was 1 μmol/l. Values are means ± SE (n = 6). **P < 0.01, ***P < 0.001.
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Fig. 4. Glibenclamide metabolism in liver is not affected by Hnf-1α deficiency. Extracts from liver, serum, and gut were prepared as described in Research Design and Methods. For chromatographic separation of glibenclamide and its metabolites, 20 µl of the sample was injected and 96 samples (0.2 ml) were collected starting at the time of injection. Radioactivity was measured by liquid scintillation counting and expressed in counts per minute. Representative chromatograms of a liver from a wild-type mouse (A) and from Hnf-1α/-- mice (B). C: Chromatogram of serum collected 5 min after intravenous injection of [3H]glibenclamide in Hnf-1α++ mouse. D: Chromatogram of a gut from Hnf-1α/-- mouse harvested 3 h after [3H]glibenclamide intravenous injection. M1, 4-trans-hydroxyglibenclamide; M2, 3-cis-hydroxyglibenclamide.

take were due to a primary effect and not secondary to a difference in serum composition between Hnf-1α++ and Hnf-1α-- mice. Furthermore, the data indicate that a decrease in hepatic transport and/or a defect in liver glibenclamide metabolism is responsible for the defect in clearance of glibenclamide in mutant Hnf-1α mice.

Glibenclamide metabolizing pathway is not altered in liver of Hnf-1α-- mice. To investigate if a defect in glibenclamide metabolism contributed to the decrease in hepatic uptake of glibenclamide, we analyzed glibenclamide and its metabolites in liver extracts. Livers from Hnf-1α++ and Hnf-1α-- mice were excised 3 h after intravenous injection of [3H]glibenclamide, and compounds were extracted and measured by HPLC analysis (Fig. 4A and B). The glibenclamide peak was identified by spiking with [3H]glibenclamide. The two metabolite peaks (4-trans-hydroxyglibenclamide and 3-cis-hydroxyglibenclamide) have been described by Rydberg et al. (18). The liver content of glibenclamide and its two major hydroxylated metabolites are shown in Table 1. Glibenclamide content was about 20-fold higher in the livers of Hnf-1α++ mice compared with Hnf-1α-- mice (158.6 ± 6.9 vs. 8.3 ± 0.7 pmol·1–1·mg–1·protein). The ratios of 4-trans-hydroxyglibenclamide (M1) and 3-cis-hydroxyglibenclamide (M2) to glibenclamide were increased in Hnf-1α-- livers (14.1 and 13.0%, respectively) compared with Hnf-1α++ mice (8.7 and 7.3%, respectively). This indicated that glibenclamide is metabolized normally in hepatocytes of Hnf-1α-- mice. Therefore, a defect in glibenclamide transport by hepatocytes is likely to be responsible for the reduced clearance of glibenclamide in the Hnf-1α-- mice.

To demonstrate that the radioactivity measured in the plasma was indeed glibenclamide and not its metabolites, a serum extract from Hnf-1α-- mice was also analyzed by HPLC 5 min after intravenous injection of [3H]glibenclamide. As shown on Fig. 4C, more than 95% of the counted radioactivity in the plasma of Hnf-1α-- mice was glibenclamide. In contrast, HPLC analysis from gut extracts obtained 3 h after intravenous injection of [3H]glibenclamide revealed the presence of predominately two hydroxylated metabolites (Fig. 4D). These data suggest that glibenclamide itself, and not its metabolites, constitutes the majority of the plasma levels in Hnf-1α-- mice. Furthermore, the presence of glibenclamide metabolites in the intestine of Hnf-1α-- mice indicates that secretion of glibenclamide metabolites into the biliary system is functional. We therefore conclude that hepatic metabolism of glibenclamide as well as biliary secretion is functional in Hnf-1α-- mice.

Discussion

In this study, we examined the role of HNF-1α in glibenclamide metabolism to study possible mechanisms for hypersensitivity to sulfonylureas in HNF-1α-dependent diabetes (MODY3). As a model system for MODY 3, we used mice in which the Hnf-1α gene was inactivated by targeted mutagenesis (4). Heterozygous Hnf-1α mice do

**TABLE 1** Absolute and relative concentrations of glibenclamide and its two hydroxylated metabolites in livers of Hnf-1α++ and Hnf-1α-- mice 3 h after intravenous injection of [3H]glibenclamide

<table>
<thead>
<tr>
<th>Liver concentration (pmol/mg protein)</th>
<th>Hnf-1α++</th>
<th>Hnf-1α--</th>
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<tbody>
<tr>
<td></td>
<td>Glibenclamide concentration</td>
<td>% total cpm</td>
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<tr>
<td>Glibenclamide</td>
<td>158.6 ± 6.9</td>
<td>84.0</td>
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<tr>
<td>4-trans-hydroxyglibenclamide</td>
<td>16.4 ± 7.0</td>
<td>8.8</td>
</tr>
<tr>
<td>3-cis-hydroxyglibenclamide</td>
<td>13.8 ± 3.3</td>
<td>7.3</td>
</tr>
</tbody>
</table>

Data are means ± SE or % and are derived from five animals for each genotype. The P values for 4-trans-hydroxyglibenclamide and 3-cis-hydroxyglibenclamide of Hnf-1α++ versus Hnf-1α-- animals were 0.04 and 0.03, respectively.
not develop diabetes, and islets have normal insulin secretion in response to glucose and other insulin secretagogues (5,19,22). Furthermore, we did not detect differences in insulin secretion or blood glucose levels after an intravenous glibenclamide infusion between wild-type and Hnf-1α−/− mice, indicating that haplo-insufficient mice are not suitable for studying the pharmacogenetics of glibenclamide in Hnf-1α deficiency. However, Hnf-1α−/− mice are diabetic, and their pancreatic islets have profound defects in glucose- and arginine-stimulated insulin secretion (19). Pancreatic islets from Hnf-1α−/− mice also exhibit significantly reduced insulin secretory responses to tolbutamide compared with Hnf-1α+/− animals in vitro (5,19). However, in vivo, we have observed that insulin responses to glibenclamide are similar in Hnf-1α−/− and Hnf-1α+/- mice. This discrepancy may be explained by the increased glibenclamide concentration to which pancreatic islets of Hnf-1α−/− animals are exposed. Therefore, this finding suggests that β-cells that are deficient in Hnf-1α are normoglycemic or hyposensitive to the actions of sulfonylureas, thus making it unlikely that a β-cell intrinsic mechanism is responsible for the increased sensitivity to sulfonylureas.

HNF-1α is expressed in the liver and the kidney—two organs that play a crucial role in the detoxification of glibenclamide. Therefore, we tested the hypothesis that hepatic metabolism is affected in Hnf-1α−/− mice. The elimination of glibenclamide from the body depends on hepatic metabolism and biliary clearance. In the liver, glibenclamide is converted into two major metabolites: 4-trans-hydroxyglibenclamide and 3-cis-hydroxyglibenclamide (18). Approximately 50% of these compounds are excreted in the urine and 50% in the bile. The first step of glibenclamide metabolism involves the transport by an as yet unknown energy-independent facilitative diffusion process (15). The uptake of glibenclamide can be inhibited by other organic anions including the unconjugated bile acid cholate, as well as loop diuretics such as bumetanide (20,23,24). We have previously shown that Hnf-1α−/− mice have elevated serum bile acid levels due to increased de novo synthesis and decreased uptake by hepatocytes (21). Therefore, the high levels of bile acids present in sera of Hnf-1α−/− mice could have contributed to the decreased clearance of glibenclamide observed in vivo. To control for these confounding factors, we studied glibenclamide uptake into cultured hepatocytes of Hnf-1α−/− and Hnf-1α+/- mice and showed that V_{max} was approximately threefold lower in mutant mice. The results demonstrate that the decreased clearance of glibenclamide is caused by a primary defect in hepatic clearance.

Inactivation of glibenclamide in the liver occurs through hydroxylation by cytochrome P450 enzymes followed by glucuronon conjugation and sulfonation (23). Thus, depending on the limiting step of glibenclamide metabolism, reduced uptake of glibenclamide into hepatocytes may be attributed to an impairment of hepatic uptake or metabolism. To investigate if hydroxylation of glibenclamide was impaired, we measured its metabolites in livers of Hnf-1α−/− and Hnf-1α+/- mice. Using HPLC analysis, we showed that the ratios of glibenclamide and its two hydroxylated metabolites 4-trans-hydroxyglibenclamide and 3-cis-hydroxyglibenclamide were significantly increased in liver extracts of Hnf-1α−/− mice compared with wild-type littermates. This result indicates that a defect in glibenclamide metabolism is not responsible for decreased glibenclamide uptake in hepatocytes of Hnf-1α−/− mice. Furthermore, the increase of the relative metabolite concentrations in liver extracts of Hnf-1α−/− mice may be due to the reduced glibenclamide concentrations in the hepatocytes of Hnf-1α−/− animals. Together, our data strongly suggest that decreased transport across the hepatocyte plasma membrane appears to be responsible for the increased biological half-life of glibenclamide in mice lacking Hnf-1α.

Petzinger and Fügkel (15) have shown that glibenclamide transport occurs through a carrier-mediated system. Several classes of transport systems have been described that mediate hepatic organic anion uptake mechanisms (25). Organic anion transporter proteins play a critical role in this process by transporting a broad spectrum of substrates in a sodium-independent manner. We have recently shown that gene expression of oatp1 (Slc21a1), Lst-1 (Slc21a6), OATPD (SLC21A11), and oat2 are downregulated or absent in livers of Hnf-1α−/− mice (21). These carriers are candidates for facilitating the transport of glibenclamide across the hepatocyte membrane. We cannot rule out that an intracellular binding protein may be involved in glibenclamide hepatic uptake as well. Meanwhile, identification of the glibenclamide transporter or intracellular binding protein remains to be established.

An example of the genetic defect altering the pharmacological response to sulfonylurea in diabetes was described 20 years ago, and a monogenic control of tolbutamide metabolism in humans was proposed (26). Our results are consistent with the mechanism described in this study. Scott and Pofferbarger (26) observed a ninefold variation in the rate of tolbutamide disappearance from plasma after intravenous administration in humans. This variation was characterized by a trimodal frequency distribution, suggestive of monogenic inheritance. The binding of tolbutamide to serum proteins was not correlated to the tolbutamide clearance. It was therefore proposed that the microsomal hepatic conversion of free tolbutamide to hydroxytolbutamide was the impaired step. However, this process can be viewed as two separate steps: first, removal of free tolbutamide from the blood by hepatocytes, and second, enzymatic conversion by hepatic microsomes. Therefore, genetic variation in genes encoding the Hnf-1α-regulated glibenclamide transporter as well as the sulfonylurea-metabolizing P450 enzymes may be responsible for differences in plasma half-lives and susceptibility to these drugs in the population.

Glibenclamide is one of the most widely used orally active sulfonylureas in the treatment of type 2 diabetes. Hypoglycemia is the most important and most often fatal adverse effect of sulfonylureas (23). We propose that hypoglycemia may occur more frequently in subjects with HNF-1α deficiency treated with glibenclamide because of a delayed disappearance of the drug from the blood. Very low doses of short-acting sulfonylureas should therefore be used initially in the treatment of MODY3 patients, and these drugs should be discontinued in subjects with liver disease. Monitoring sulfonylurea plasma concentration...
in MODY3 patients may also be useful for avoiding hypoglycemia.

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