Interaction of Oral Antidiabetic Drugs With Hepatic Uptake Transporters

Focus on Organic Anion Transporting Polypeptides and Organic Cation Transporter 1

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OBJECTIVE—The uptake of drugs into hepatocytes is a key determinant for hepatic metabolism, intrahepatic action, their subsequent systemic plasma concentrations, and extrahepatic actions. In vitro and in vivo studies indicate that many drugs used for treatment of cardiovascular diseases (e.g., oral antidiabetic drugs, statins) are taken up into hepatocytes by distinct organic anion transporters (organic anion transporting polypeptides [OATPs]; gene symbol SLCO/SLC21) or organic cation transporters (OCTs; gene symbol SLC22). Because most patients with type 2 diabetes receive more than one drug and inhibition of drug transporters has been recognized as a new mechanism underlying drug-drug interactions, we tested the hypothesis of whether oral antidiabetic drugs can inhibit the transport mediated by hepatic uptake transporters.

RESEARCH DESIGN AND METHODS—Using stably transfected cell systems recombinantly expressing the uptake transporters OATP1B1, OATP1B3, OATP2B1, or OCT1, we analyzed whether the antidiabetic drugs repaglinide, rosiglitazone, or metformin influence the transport of substrates and drugs (for OATPs, sulfobromophthalein [BSP] and pravastatin; for OCT1, 1-methyl-4-phenylpyridinium [MPP^+] and metformin).

RESULTS—Metformin did not inhibit the uptake of OATP and OCT1 substrates. However, OATP-mediated BSP and pravastatin uptake and OCT1-mediated MPP^+ and metformin uptake were significantly inhibited by repaglinide (half-maximal inhibitory concentration [IC_{50}] 1.6–5.6 μmol/l) and rosiglitazone (IC_{50} 5.2–30.4 μmol/l).

CONCLUSIONS—These in vitro results demonstrate that alterations of uptake transporter function by oral antidiabetic drugs have to be considered as potential mechanisms underlying drug-drug interactions. ***Diabetes*** **57:1463–1469, 2008**

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BSP, sulfobromophthalein; HEK293 cell, human embryonic kidney cell; IC_{50}, half-maximal inhibitory concentration; MDCKII cell, Madin-Darby canine kidney cell; MPP^+, 1-methyl-4-phenylpyridinium; OATP, organic anion transporting polypeptide; OCT, organic cation transporter.

Patients with type 2 diabetes are commonly treated with more than one drug. It is therefore essential to understand mechanisms underlying drug-drug interactions, which might cause changes in the pharmacokinetics and effects of these drugs. It is now well established that drug transporters are major determinants of drug disposition and effects (1,2). Moreover, inhibition or induction of drug transporters are newly recognized mechanisms of drug-drug interactions (3–5).

Oral antidiabetic drugs need to be taken up from the portal venous blood via the basolateral membrane into hepatocytes before they are metabolized in the cell, cause drug effects via intrahepatic mechanisms, or are further transported back into the systemic circulation for extrahepatic effects (6–9). Recent in vitro and in vivo studies highlighted that the transporter-mediated uptake of oral antidiabetic drugs is an important determinant for their disposition and effects (9–11). These uptake mechanisms are also important for disposition and action of a broad variety of cardiovascular drugs frequently used concomitantly in patients with type 2 diabetes (e.g., statins, ACE inhibitors, and angiotensin type 1 receptor blockers) (12).

This study focuses on uptake transporters belonging to the organic anion transporting polypeptide (OATP; gene symbol SLCO/SLC21) and organic cation transporter (OCT; gene symbol SLC22) families. Members of the OATP family transport a variety of anionic endogenous substances and drugs, including hydroxymethylglutaryl-CoA reductase inhibitors, such as fluvastatin, pitavastatin, pravastatin, and rosuvastatin (12,13). Furthermore, it has been shown that glitazones and repaglinide may be substrates for the hepatocyte-specific OATP family member OATP1B1 (9,14). OATP1B1, OATP1B3, and OATP2B1 are localized in the basolateral membrane of human hepatocytes mediating the uptake of endogenous substances and drugs from the portal venous blood (15–17).

Members of the OCT family play essential roles in the handling of cationic drugs and endogenously synthesized organic cations. Human OCT1 is expressed primarily in the liver, localized in the basolateral membrane of hepatocytes mediating the hepatic uptake of several cationic drugs (e.g., metformin). In addition, various drugs (e.g., cimetidine, desipramine, midazolam, citalopram, and clonidine) have been identified to inhibit OCT1-mediated uptake (18).

Despite the increasingly recognized role of hepatic uptake transporters for drug disposition, it has not been

TABLE 1
Comparison of pharmacokinetic data of oral antidiabetic drugs in humans with IC_{50} values of OATP1B1-, OATP1B3-, and OATP2B1-mediated BSP uptake and OCT1-mediated MPP^+ uptake obtained in the present study

	Dosage (mg)	Cmax (mg/l)	Cmax (μmol/l)	Cport. vn. (μmol/l)	IC_{50} OATP1B1 (μmol/l)	IC_{50} OATP1B3 (μmol/l)	IC_{50} OATP2B1 (μmol/l)	IC_{50} OCT1 (μmol/l)	References
Metformin	1 × 850	5.9	35.9	356.7	>100 (BSP)	>100 (BSP)	>100 (BSP)	>1,000 (MPP^+)	41
Repaglinide	1 × 4	0.1	0.2	0.8	2.2 (BSP) (pravastatin)*	5.6 (BSP) (pravastatin)*	5.2 (BSP)	1.8 (MPP^+) 1.6 (metformin)	42
Rosiglitazone	1 × 8	0.8	1.7	2.7	6.0 (BSP) (pravastatin)†	10.9 (BSP) (pravastatin)†	5.2 (BSP)	30.4 (MPP^+) 6.9 (metformin)	43

IC_{50} data are derived from the measurements shown in Figures 3–8; respective tested substrates are indicated in parentheses. *IC_{50} value was not determined, but there is an inhibition at all inhibitor concentrations investigated. †IC_{50} value was not determined, but there is an inhibition at the highest inhibitor concentration tested. Cmax, maximal plasma concentration in the systemic circulation; Cport.vn., predicted concentration in portal venous blood (according to Ito et al. [35]).

studied systematically whether the oral antidiabetic drugs repaglinide, rosiglitazone, and metformin, which have previously been shown to interact with OATPs or OCTs (9,14,18), are inhibitors of hepatic uptake transport proteins. To gain more insights into the potential role of uptake transporters for antidiabetic drug–mediated drug-drug interactions, we used cell systems stably expressing the human uptake transporters OATP1B1, OATP1B3, OATP2B1, or OCT1 and tested the influence of these oral antidiabetic drugs on the uptake of OATP substrates and OCT1 substrates.

RESEARCH DESIGN AND METHODS

Chemicals and antibodies. [^{3}H]sulfobromophthalein ([^{3}H]BSP; 7,585 GBq/mmol) was obtained from Hartmann Analytic (Braunschweig, Germany). [^{3}H]1-methyl-4-phenylpyridinium ([^{3}H]MPP^+; 85 Ci/mmol), [^{3}H]rosiglitazone (50 Ci/mmol), and unlabeled rosiglitazone were obtained from BIOTREND Chemikalien (Cologne, Germany). Unlabeled metformin and poly-D-lysine hydrobromide were purchased from Sigma-Aldrich Chemie (Taufkirchen, Germany). Unlabeled repaglinide was obtained from Chemos (Regenstauf, Germany). [^{14}C]metformin hydrochloride (0.1 Ci/mmol) was provided by Merck (Darmstadt, Germany). Methanol (hypergrade), *n*-hexane (pro-analysis), acetonitrile (hypergrade), and acetic acid (supra-pure) were purchased from Merck. Diethyl ether (99.8% purity), ammonium acetate (pro-analysis), and ibuprofen were obtained from Sigma-Aldrich Chemie.

Cell culture. Stably transfected human embryonic kidney cells (HEK293 cells) and Madin-Darby canine kidney cells (MDCKII cells) were cultured as described previously (5,19). For uptake and inhibition experiments, stably transfected HEK293 cells recombinantly expressing human OATP1B1 (HEK-OATP1B1), human OATP1B3 (HEK-OATP1B3) (5), or human OATP2B1 (HEK-OATP2B1) and the respective HEK control cells (HEK-Co/418, transfected with the empty expression vector pcDNA3.1; or HEK-Co/Hy, transfected with the empty expression vector pcDNA3.1/Hygro) were seeded in 0.1 mg/ml poly-D-lysine–coated 12-well plates (Cell Culture Multiwell Plate Cellstar; Greiner Bio-One, Frickenhausen, Germany) at an initial density of 700,000 cells/well. The cells were grown to confluence for 1 day and then induced with 10 mmol/l sodium butyrate (Merck) for 24 h before the uptake experiments to obtain higher levels of the recombinant protein (20).

MDCKII cells were transfected with the respective plasmid pcDNA3.1/Hygro(−)-OCT1 containing the full-length cDNA encoding the human OCT1-protein (NM_003057) using Effectene transfection reagent (Qiagen, Hilden, Germany). After hygromycin (800 μg/ml) selection, single colonies were characterized for *SLC22A1* (encoding human OCT1) mRNA expression by real-time PCR analysis. Vector transfected MDCKII control cells were estab-

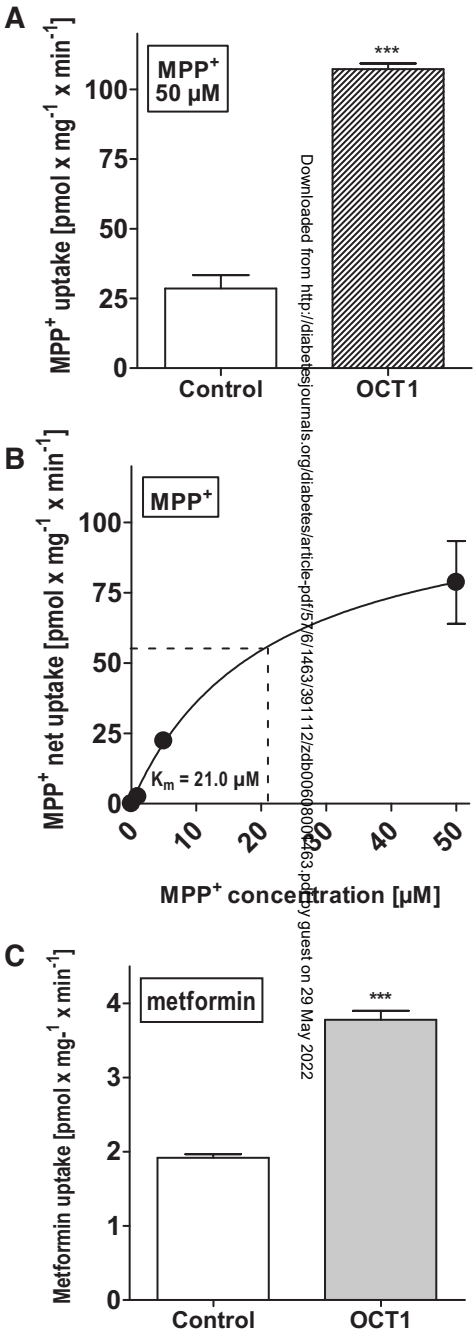

FIG. 1. Characterization of stably transfected MDCK cells. *A*: Intracellular MPP^+ accumulation in MDCKII-OCT1 and MDCKII-control (Control) cells after 10-min incubation with 50 μmol/l MPP^+. *B*: Net intracellular MPP^+ accumulation in MDCKII-OCT1 cells after 10-min incubation with increasing MPP^+ concentrations. The uptake was obtained by subtracting the uptake in vector-transfected cells (control) from that in OCT1-expressing cells. K_m and V_{max} values were calculated by fitting the data to a one-site binding curve. *C*: Intracellular metformin accumulation in MDCKII-OCT1 and MDCKII-control (Control) cells after 10-min incubation with 10 μmol/l metformin. Each value is the mean ± SE (n = 3). ***P < 0.001 vs. control.

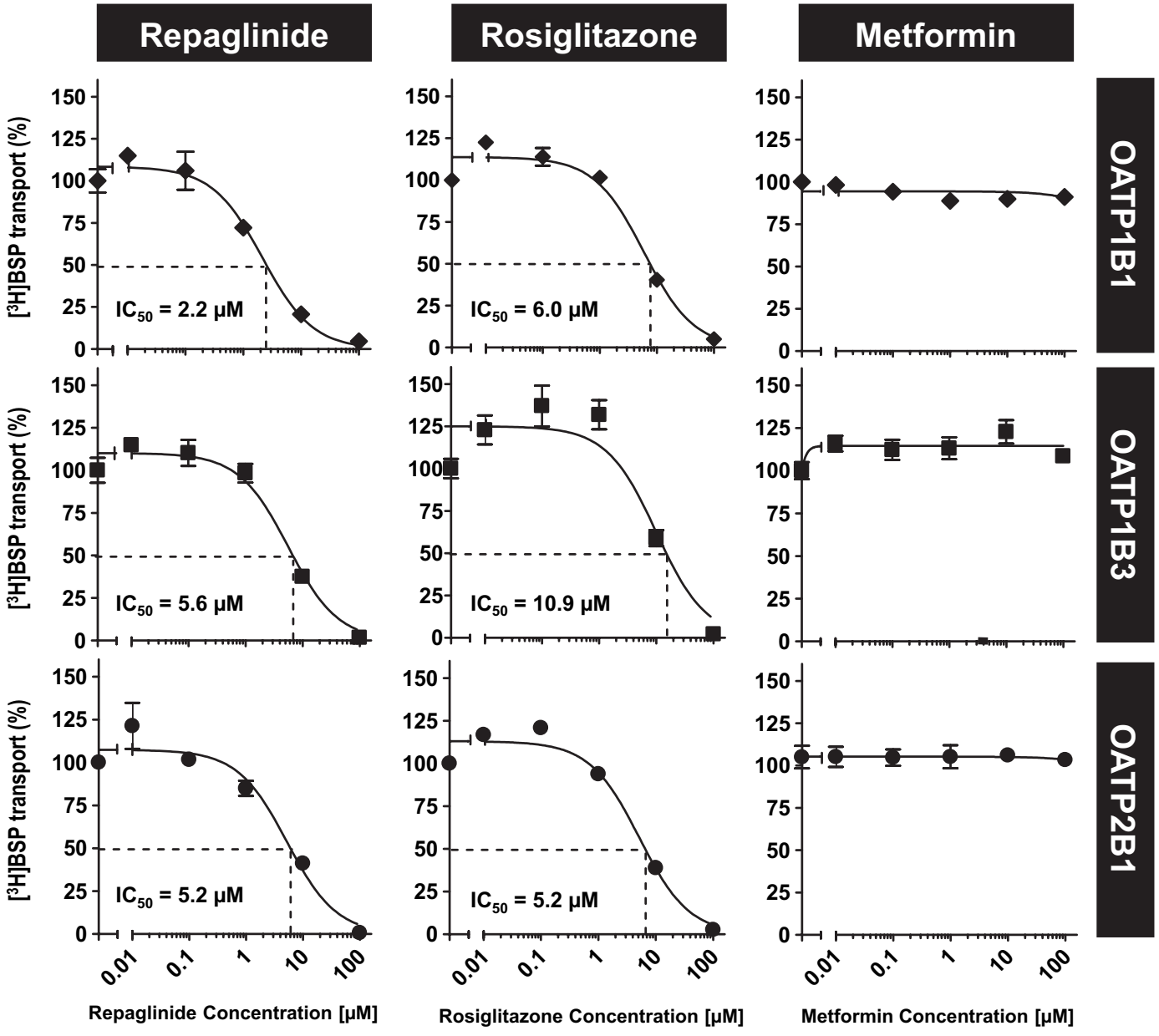

FIG. 2. Inhibition of OATP-mediated BSP uptake by oral antidiabetic drugs. Inhibitory effect of repaglinide, rosiglitazone, and metformin on OATP1B1-, OATP1B3-, and OATP2B1-mediated BSP uptake after 10-min incubation. IC_{50} values were calculated by fitting the data to a sigmoidal dose-response regression curve. Data are shown as the percentage of the mediated BSP uptake in the absence of oral antidiabetic drugs. Each value is the mean ± SE (n = 3).

lished by the same method using the respective expression plasmid without insert for transfection.

For MPP^+ uptake experiments, MDCKII cells were seeded in 0.1 mg/ml poly-D-lysine–coated 12-well plates (Cell Culture Multiwell Plate Cellstar; Greiner Bio-One) at an initial density of 700,000 cells per well. The cells were grown to confluence for 2 days and induced with 10 mmol/l sodium butyrate (Merck) for 24 h before the uptake experiments (20).

Uptake assays. Before starting the uptake experiments with the HEK cells, cells were washed with prewarmed (37°C) uptake buffer (142 mmol/l NaCl, 5 mmol/l KCl, 1 mmol/l K_2HPO_4, 1.2 mmol/l $MgSO_4$, 1.5 mmol/l $CaCl_2$, 5 mmol/l glucose, and 12.5 mmol/l HEPES, pH 7.3). [^{3}H]BSP was dissolved in uptake buffer, and unlabeled BSP was added to the final concentration of 0.05 μmol/l BSP for studies with HEK-OATP1B1 and 1 μmol/l BSP for studies with HEK-OATP1B3 and HEK-OATP2B1, respectively. To characterize repaglinide and rosiglitazone as inhibitors of OATP-mediated uptake, both drugs were added in increasing concentrations (0.01–100 μmol/l). The cells were incubated with the solution at 37°C for 10 min as described previously (5). Afterward, the cells were washed three times with ice-cold uptake buffer before lysing the cells with 0.2% SDS. The intracellular accumulation of radioactivity was detected by liquid scintillation counting (Perkin Elmer Life Sciences). For the experiments using pravastatin as substrate for OATP1B1 and OATP1B3, a concentration of 50 μmol/l pravastatin was used. To test the inhibitory effect of repaglinide and rosiglitazone, each drug was added in concentrations of 10 and 100 μmol/l. The uptake assay was performed as described above. The intracellular pravastatin concentration was determined by liquid chromatography with tandem mass spectrometry detection analysis as previously described (5).

The uptake experiments with MDCKII-OCT1 cells were carried out in an analogous manner. [^{3}H]MPP^+ was dissolved in uptake buffer, and unlabeled MPP^+ was added to the final concentration of 30 μmol/l. To characterize repaglinide, rosiglitazone, and metformin as inhibitors of OCT1-mediated uptake, the drugs were added in increasing concentrations of 0.1–1,000 μmol/l for metformin and 0.1–100 μmol/l for repaglinide and rosiglitazone, respectively. For experiments using metformin as substrate of OCT1, [^{14}C]metformin was dissolved in uptake buffer, and unlabeled metformin was added to a final concentration of 10 μmol/l. To test the inhibitory effect of repaglinide and rosiglitazone, each drug was added in a concentration of 0.1–100 μmol/l. All experiments were repeated at least three times.

Data analysis. The OATP1B1-, OATP1B3-, OATP2B1-, and OCT1-mediated net uptake was obtained by subtracting the uptake in vector-transfected cells

from that in OATP1B1-, OATP1B3-, OATP2B1-, and OCT1-expressing cells. The percentage of uptake inhibition was calculated from control experiments in the absence of antidiabetic drugs (100% uptake). The corresponding half-maximal inhibitory concentration (IC_{50}) values for uptake inhibition were calculated by fitting the data to a sigmoidal dose-response regression curve (Prism 4.01 2004; GraphPad Software, San Diego, CA). Effects of repaglinide and rosiglitazone on pravastatin uptake in OATP1B1 and OATP1B3 cells were analyzed with unpaired two-tailed *t* test (Prism 4.01 2004; GraphPad Software). A value of $P < 0.05$ was required for statistical significance.

RESULTS

Characterization of MDCKII-OCT1 cells. To examine the inhibitory potency of antidiabetic drugs on OCT1-mediated uptake, MDCKII cells were stably transfected with the *SLC22A1* cDNA and selected for a high expression of the uptake transporter OCT1. The *SLC22A1* mRNA expression of the selected cell clones was analyzed using quantitative real-time PCR. This analysis demonstrated a high *SLC22A1* mRNA expression in several MDCKII-OCT1 clones compared with vector-transfected cells. The cell clone with the highest mRNA expression was used for further transport experiments using the prototypic tritium-labeled substrate [^{3}H]MPP^+. MPP^+ was shown to be a high-affinity substrate for OCT1 with a K_m value of ~21 μmol/l, which is in accordance with previously published data (18). The uptake experiments (Fig. 1*A* and *B*) demonstrated that MDCKII-OCT1 cells were able to mediate uptake of MPP^+ (50 μmol/l) into cells with an uptake ratio of 3.7-fold compared with control cells. Furthermore, we confirmed that metformin, previously shown to be a substrate for OCT1 with a K_m value of 1,470 μmol/l (18), is also transported by the newly established MDCKII-OCT1 cells (Fig. 1*C*).

Inhibition of OATPs and OCT1 by antidiabetic drugs. The results of the inhibition experiments are summarized in Table 1 and Figs. 2–4. Metformin did not inhibit OATP1B1-, OATP1B3-, or OATP2B1-mediated BSP uptake (Fig. 2) or uptake of the OCT1 substrate MPP^+ (Fig. 4). However, OATP-mediated BSP and pravastatin uptake and OCT1-mediated MPP^+ and metformin uptake were significantly inhibited by repaglinide (Figs. 2–4; Table 1) and rosiglitazone (Figs. 2–4; Table 1).

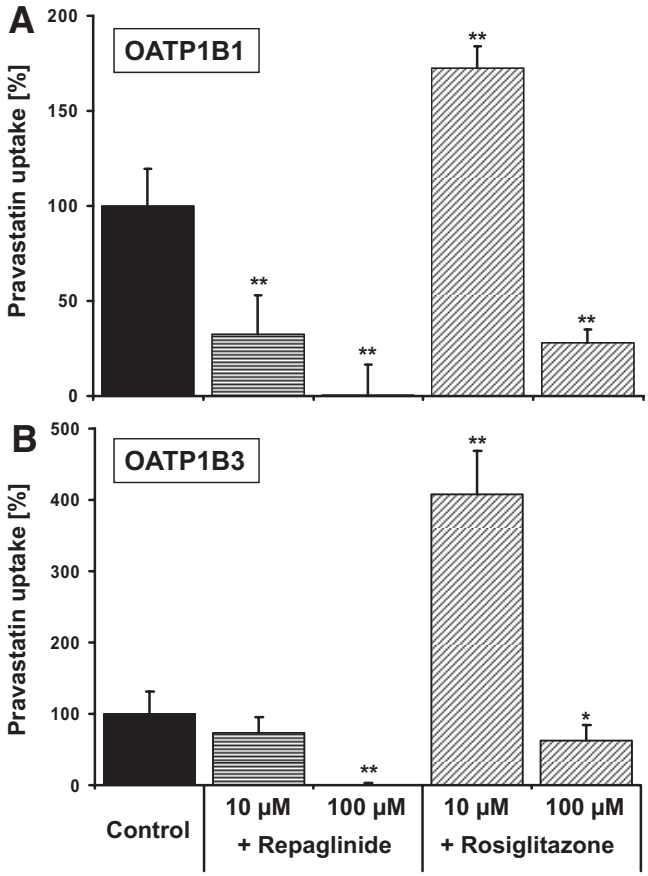

FIG. 3. Inhibition of the OATP1B1- and OATP1B3-mediated pravastatin uptake by repaglinide and rosiglitazone. Inhibitory effect using 10 and 100 μmol/l concentrations of the oral antidiabetic drugs repaglinide and rosiglitazone on OATP1B1-mediated (*A*) and OATP1B3-mediated (*B*) 50 μmol/l pravastatin uptake after 10-min incubation. Data are shown as the percentage of the transporter-mediated pravastatin uptake in the absence of oral antidiabetic drugs (Control). Each value is the mean ± SE ($n = 3$). *$P < 0.05$; **$P < 0.01$ vs. control.

DISCUSSION

Patients with type 2 diabetes frequently have to be treated with more than one drug. Effects of oral antidiabetic drugs depend on the extent of drug absorption from the gut lumen, on metabolism of the drug in the liver, and on the extent of its excretion into bile and urine (21). In general, modification of all these processes by a second, concomitantly administered drug can alter the effects of oral antidiabetic drugs (21).

Recently, it was recognized that a broad variety of drugs, including many cardiovascular drugs such as statins and angiotensin II-receptor antagonists, is transported through biological membranes via specific transport proteins (15,22–24). For example, the efflux transporter P-glycoprotein, which translocates its substrates from the inside of the cell to the outside (e.g., from the hepatocyte into bile) is a major determinant of drug effects (1). If P-glycoprotein–mediated drug excretion is inhibited by a second, concomitantly administered compound, drug plasma concentrations increase significantly and may result in drug toxicity (3).

In addition to efflux transporters (e.g., P-glycoprotein), uptake transporters such as the OATPs or the OCTs are important for drug disposition. For example, they mediate the uptake of multiple cardiovascular drugs from the portal venous blood into the hepatocytes (12,18). This transport process is an essential prerequisite for subsequent drug metabolism in the hepatocytes.

The goal of our in vitro study was to investigate whether the oral antidiabetic drugs repaglinide, rosiglitazone, and metformin are inhibitors of the hepatic uptake transporters of the OATP family (OATP1B1, OATP1B3, and OATP2B1) and of the OCT family member OCT1. Whereas metformin did not affect the function of hepatic uptake transporters, both repaglinide and rosiglitazone were significant inhibitors of organic anion and organic cation transport. The following clinical consequences can result from the observed interaction with repaglinide and rosiglitazone. First, if hepatic uptake of a drug (e.g., pravastatin) is inhibited by repaglinide and rosiglitazone and possibly by further oral antidiabetic drugs, the first step (i.e., uptake into hepatocytes) of biliary drug elimination is blocked. This causes increased plasma concentrations and an increased risk of adverse drug reactions. The importance of an impaired uptake transporter function is convincingly shown for OATP1B1. One polymorphism (c.521T>C) in the gene encoding for OATP1B1 results in a significantly reduced OATP1B1 function (25), a situation that is comparable to the OATP1B1 function in the pres-

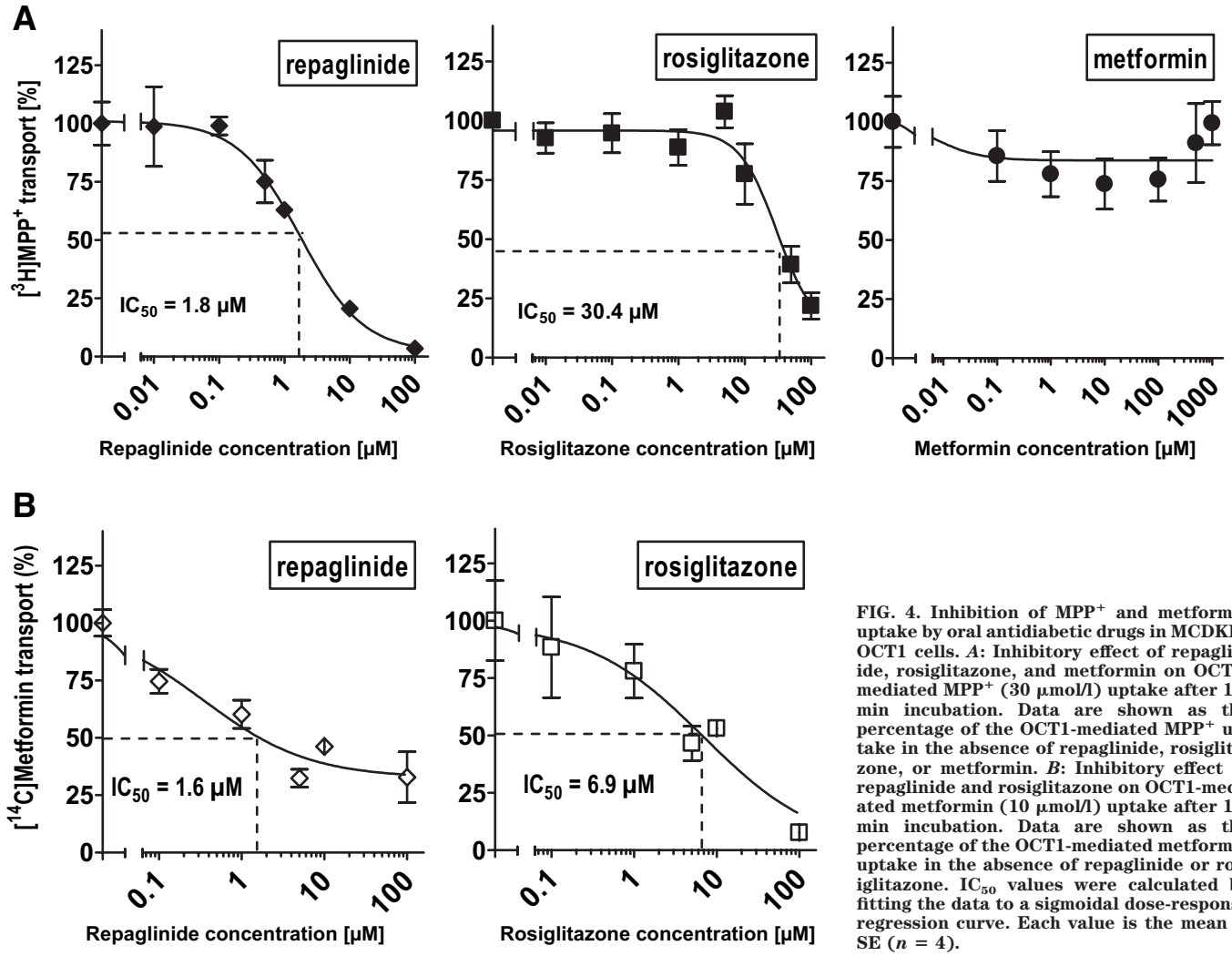

FIG. 4. Inhibition of MPP^+ and metformin uptake by oral antidiabetic drugs in MCDKII-OCT1 cells. *A*: Inhibitory effect of repaglinide, rosiglitazone, and metformin on OCT1-mediated MPP^+ (30 μmol/l) uptake after 10-min incubation. Data are shown as the percentage of the OCT1-mediated MPP^+ uptake in the absence of repaglinide, rosiglitazone, or metformin. *B*: Inhibitory effect of repaglinide and rosiglitazone on OCT1-mediated metformin (10 μmol/l) uptake after 10-min incubation. Data are shown as the percentage of the OCT1-mediated metformin uptake in the absence of repaglinide or rosiglitazone. IC_{50} values were calculated by fitting the data to a sigmoidal dose-response regression curve. Each value is the mean ± SE (n = 4).

ence of drugs, which inhibit this uptake transporter. Several studies showed that subjects carrying this polymorphism with the reduced uptake transporter function have significantly higher plasma concentrations of pravastatin (26–29). Interestingly, for other drugs, no influence of this genetic variation could be observed (30). This could be due to an overlapping substrate spectrum with OATP1B3, possibly compensating for a reduced uptake mediated by a mutated OATP1B1 protein.

A second clinical consequence of decreased hepatic drug uptake is a reduced effect of the affected drug (e.g., pravastatin). This option applies if the drug effect (e.g., for pravastatin, cholesterol-lowering effect) is mediated via mechanisms within the hepatocyte. A genetically determined reduced hepatic uptake of pravastatin was recently shown to cause a reduced effect of pravastatin (31–33).

The third possible clinical consequence of inhibition of uptake transporter function is increased extrahepatic effects (e.g., for statins, an increased risk of myotoxicity). This reduced benefit (cholesterol-lowering effect)-to-risk (myotoxicity) ratio for OATP1B1-dependent statins by inhibition of hepatic uptake was recently highlighted in an excellent review by Neuvonen et al. (34).

It should be noted that our study focused on in vitro studies on mechanisms of drug interactions with oral antidiabetic drugs. As outlined below, the clinical consequences (e.g., pharmacodynamic consequences) are difficult to predict and require further clinical studies. Another interesting observation of our study was the unexpected stimulation of OATP1B3-mediated pravastatin uptake by rosiglitazone (Fig. 3). Similar observations have been made for other substrates (5); however, the molecular mechanisms underlying this effect are unclear and require further studies.

As drugs reach the portal vein directly after intestinal absorption, the drug concentration in portal venous blood is higher than in the systemic circulation. For estimation of the drug concentrations in portal venous blood, we used the method of Ito et al. (35) (Table 1). The predicted portal venous blood concentrations for repaglinide and rosiglitazone are in the same low micromolar range as the determined IC_{50} values for inhibition of hepatic uptake, suggesting that inhibition of hepatic drug transporters might cause clinically relevant drug-drug interactions in humans.

To the best of our knowledge, no clinical studies have, so far, been conducted to investigate an impact of repaglinide or rosiglitazone on disposition of pravastatin or other OATP substrates in humans. Pharmacodynamic interactions between metformin and rosiglitazone (36,37) or repaglinide (38) in patients have intensively been investigated. For both rosiglitazone and repaglinide, additional effects on glucose metabolism in patients with type 2 diabetes receiving simultaneously metformin have been

shown. Whether these effects can in part be explained by an interaction with hepatic metformin uptake is presently unclear. One study examined the pharmacokinetic interaction between rosiglitazone and metformin in humans (39), however, without a significant pharmacokinetic interaction between these drugs.

The question arises of whether our findings on inhibition of uptake transporter function by oral antidiabetic drugs can be used to draw general conclusions regarding drug-drug interactions. Similar to the present data, macrolid antibiotics were previously shown to cause a relevant inhibition of hepatic uptake transporters (5). Moreover, there is evidence that other oral antidiabetic drugs (glibenclamide) are inhibitors of hepatic uptake transporters (40). Thus, our in vitro data generated in this study indicate that inhibition of uptake transporters by oral antidiabetic drugs is a newly recognized mechanism of potential drug-drug interactions. Our data provide the basis for controlled clinical studies to clarify the importance of drug-drug interactions with oral antidiabetic drugs in studies with healthy volunteers and patients.

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