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OATP1B3 Is Expressed in Pancreatic β-Islet Cells and Enhances the Insulinotropic Effect of the Sulfonylurea Derivative Glibenclamide

PHARMACOLOGY AND THERAPEUTICS

Organic anion transporting polypeptide OATP1B3 is a membrane-bound drug transporter that facilitates cellular entry of a variety of substrates. Most of the previous studies focused on its hepatic expression and function in hepatic drug elimination. In this study, we report expression of OATP1B3 in human pancreatic tissue, with the abundance of the transporter localized in the islets of Langerhans. Transport studies using OATP1B3-overexpressing MDCKII cells revealed significant inhibition of the cellular uptake of the known substrate cholecystokinin-8 in the presence of the insulinotropic antidiabetes compounds tolbutamide, glibenclamide, glimepiride, and nateglinide and identified glibenclamide as a novel substrate of OATP1B3. Sulfonylurea derivatives exert their insulinotropic effect by binding to the SUR1 subunit of the K_{ATP} channels inducing insulin secretion in β-cells. Here, we show that transient overexpression of human OATP1B3 in a murine β-cell line (MIN6)—which exhibits glucose and glibenclamide-sensitive insulin secretion—significantly enhances the insulinotropic effect of glibenclamide without affecting glucose-stimulated insulin secretion. Taken together, our data provide evidence that the drug transporter OATP1B3 functions as a determinant of the insulinotropic effect of glibenclamide on the tissue level. Changes in transport activity based on drug-drug interactions or genetic variability may therefore influence glibenclamide efficacy.

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The organic anion transporting polypeptides (OATPs/ gene *SLCO*) belong to the solute carrier (SLC) superfamily comprising membrane-bound transporters facilitating cellular entry and are characterized by a wide range of diverse endogenous and exogenous substrates. Of the human OATPs, OATP1B1 (also OATP-C) has been studied most extensively. This transporter is expressed exclusively at the basolateral membrane of hepatocytes and thought to mediate hepatic uptake of substrate drugs. OATP1B1 is highly polymorphic; a well-characterized genetic variant, c.521T>C, has been associated with aberrant cell-surface trafficking and reduced function of the

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transporter in vitro (1), translating into reduced hepatic uptake and pronounced changes in drug disposition. In detail, reduced hepatic OATP1B1-mediated uptake results in augmented plasma levels of substrate drugs (summarized in 2). It is now widely accepted that OATP1B1 plays a significant role in the pharmacokinetics of many clinically relevant drugs including statins, irinotecan and atrasentan, and repaglinide (3). OATP1B3, the second member of the OATP1B subfamily, was cloned and functionally tested a decade ago (4), but little is known about its clinical significance. This transporter has been reported to be expressed in human liver (5) and placenta (6) as well as various tumors (7–10).

Using an Oatp1b2 knockout mouse model, we observed dysregulation of cholesterol and glucose homeostasis in animals that lack the expression of Oatp1b2, the murine ortholog of the human OATP1B transporters, compared with wild-type animals. This phenotype in mice is most likely based on reduced hepatic uptake and therefore activity of thyroid hormones (11). Concerning glucose homeostasis, it is prerequisite to investigate effects on the endocrine pancreas, particularly with respect to insulin secretion. However, we did not observe changes in expression of Glut2 (Slc2a2) in mouse pancreas, which we found to be highly downregulated in liver. Importantly, we did not observe expression of Oatp1b2 in murine pancreas. In humans, OATP1B3 expression has been initially reported in pancreatic tumors (12) and, more recently, also in healthy human pancreas, though with significant interindividual variability (10,13). Furthermore, recent studies suggested an interaction of OATP1B3 with oral antidiabetes medication including the sulfonylurea derivatives glimepiride and glibenclamide, as well as the glinids nateglinide and repaglinide (14–16). In order to exert their antidiabetes effects, these compounds target the sulfonylurea receptor 1 (SUR1), which is highly expressed in pancreas. The presence of OATP1B3 in pancreatic tissue and its reported interaction with insulinotropic antidiabetes agents raised the question whether the transporter is expressed in the human islets of Langerhans and whether it influences the insulinotropic efficacy of oral antidiabetes agents.

RESEARCH DESIGN AND METHODS

Tissue Samples

The human healthy pancreatic, renal, placental, and hepatic tissue samples (mRNA, protein, and paraffin-embedded tissue sections) were commercially obtained from BioCat GmbH (German distributor of Biochain, Heidelberg, Germany).

Cell Lines and Cell Culture

HeLa (ACC-57) and MDCKII (American Type Culture Collection no. CRL-2936) cells were originally obtained from the DSMZ-German Collection of microorganism and cell culture (Leibnitz Institute, Braunschweig, Germany) and American Tissue Culture Collection, respectively. The MDCKII cells were the basis for the generation of the stably transfected cell lines MDCKII-OATP1B3 and MDCKII-OATP1B1 using the coding sequences of both transporters subcloned into the retroviral expression vector pQCXIN (Clontech, Saint-Germain-en-Laye, France). MIN6 (17) cells were kindly provided by Dr. Jun-Ichi Miyazaki (Osaka University, Osaka, Japan). All cells were cultured in Dulbecco's modified Eagle's medium (DMEM), supplemented with 10% FCS, and 2 mmol/L L-glutamine (PAA, Coelbe, Germany) at 37°C in a humidified atmosphere containing 5% CO_2.

Real-Time PCR

The level of mRNA expression of SLCO1B3 (OATP1B3), SLCO1B1 (OATP1B1), and 18S rRNA was determined using the predeveloped gene expression assays Hs00251986_m1, Hs00272374_m1, and Hs99999901_s1, respectively (Life Technologies, Darmstadt, Germany). Human mRNA tissue samples, commercially obtained from BioCat GmbH, were reverse transcribed using the High Capacity cDNA Reverse Transcription kit (Life Technologies). Subsequently, 20 ng cDNA was used for real-time PCR using the TaqMan7900 HT (Life Technologies) with a standardized cycling protocol. PCR products were visualized using a 2.5% agarose gel electrophoresis.

Immunohistochemistry

Paraffin-embedded tissue sections were obtained from BioCat GmbH. After deparaffinization with xylol, the sections were rehydrated in a decreasing alcohol series and treated with heat-induced epitope retrieval (0.1 mol/L citrate buffer, pH 6.0). Cells were cultured on coverslips and then fixed in 4% paraformaldehyde. Subsequently, the slides or coverslips were washed in PBS and blocked with 5% FBS for 1 h. Tissue sections were incubated overnight at 4°C with a custom-made anti-OATP1B3 antibody (1:100) (5) (rabbit, kindly provided by Richard Kim, University of Western Ontario, London, ON, Canada), an anti-insulin antibody (ab7842, guinea pig; Abcam, Cambridge, MA), or an anti-glucagon antibody (ab8055, rabbit; Abcam). After several washing steps with PBS, the slides were incubated for 1.5 h at room temperature with the respective fluorescence-labeled secondary antibody (Life Technologies) diluted 1:250 and mounted in mounting medium supplemented with the nuclei stain DAPI after washing with PBS (Carl Roth GmbH, Karlsruhe, Germany). Fluorescent staining was detected using an Axio Observer.D1 microscope (Carl Zeiss GmbH, Jena, Germany).

Adenoviral Infection

For transient overexpression, adenoviral vectors were generated containing the coding sequence of OATP1B3 or of enhanced GFP (eGFP) as a control. Briefly, the coding sequences was amplified by PCR and subcloned

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into pENTR1A (Invitrogen, Life Technologies). In the case of OATP1B3, a PacI restriction site was mutated using the following primer: 5′-ACATCAAGTTTAT-CAACCTGTTTGATTAATCAAACCTTATCATTC-′3; the introduced mutation is not coding. After sequence control by Sanger Sequencing, the coding sequences were transferred into the pAD-V5/DEST vector by directed recombination using the LR clonase (Invitrogen, Life Technologies). The PacI digested plasmid was transfected into HEK293A cells using Lipofectamine 2000 (Invitrogen) for virus production as described by the manufacturer (Invitrogen, Life Technologies). After virus enrichment, an MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] plaque-forming assay was performed. For further studies, the adenoviruses Ad-OATP1B3, Ad-LacZ, and Ad-eGFP were used for infection of HeLa and MIN6 cells, respectively. In detail, 24 h after seeding the cells were infected with the adenovirus diluted in Opti-MEM (Invitrogen). The medium was changed 6 h after infection, and the cells were incubated for a further 48 h before starting the respective experiments.

Transport Studies

For transport studies, MDCKII cells were treated with 500 μmol/L butyrate 24 h prior to the experiment. After incubation with the adenovirus (transiently expressing cells) or with butyrate (stably expressing cells), cells were washed several times with prewarmed PBS and then incubated for the respective time (5 min for inhibition studies) with the radiolabeled compounds diluted in incubation buffer containing 142 mmol/L NaCl, 5 mmol/L KCl, 1 mmol/L KH_2PO_4, 1.5 mmol/L $CaCl_2$, 1.2 $MgSO_4$, 5 mmol/L glucose, and 12.5 mmol/L HEPES). Uptake was determined in the presence and absence of potential inhibitors. The compounds tested for inhibition, namely, nateglinide, tolbutamide, glibenclamide, and glimepiride, were obtained from Sigma-Aldrich (Kaufbeuren, Germany). After incubation, the cells were washed three times with ice-cold PBS and lysed in lysis puffer containing 0.2% SDS and 5 mmol/L EDTA. Cellular accumulation of radiolabeled [^{3}H]cholecystokinin-8 (CCK-8) (PerkinElmer), [^{3}H]glibenclamide (PerkinElmer), and [^{3}H]bromosulfophthalein (Hartmann Analytic, Braunschweig, Germany) was determined using liquid scintillation fluid (Rotiszint eco plus; Roth, Karlsruhe, Germany) and a scintillation β-counter (type 1409; LKB-Wallac/PerkinElmer, Freiburg, Germany). In addition, an aliquot of the lysate was used for protein quantification.

Determination of Insulin Secretion

During the 48 h after adenoviral infection, MIN6 cells were cultured in Dulbecco's modified Eagle's medium containing 25 mmol/L glucose supplemented with 15% FCS. For the insulin secretion assay, the cells were first rinsed three times with the low-glucose (2.8 mmol/L) Krebs buffer (115 mmol/L NaCl, 4.7 mmol/L KCl, 1 mmol/L $MgSO_4$, 1.2 mmol/L KH_2PO_4, 25 mmol/L $NaHCO_3$, 1 mmol/L sodium pyruvate, and 10 mmol/L HEPES, pH 7.4) and then incubated for 2 h with the low-glucose Krebs buffer for equilibration. After that, the cells were incubated for an additional 1 h for collection of the basal supernatant, followed by 1 h incubation with Krebs buffer containing glibenclamide or high glucose (16.7 mmol/L) for collection of the stimulated supernatant, respectively. Insulin content was determined using the Ultra Sensitive Mouse Insulin ELISA (Chrystal Chem, Downers Grove, IL) following the manufacturer's instructions. Absorbance was measured after 10 min incubation using a plate reader (Tecan infinite M200; Crailsheim, Germany).

Statistical Analysis

Experimental data are expressed as mean ± SD. Data analysis was performed using the GraphPad Prism Software (GraphPad Software, San Diego, CA). For statistical analysis, the Student *t* test was used. Parameters for saturation kinetics (V_{max} and K_m) were estimated by nonlinear curve fitting using Prism. The half-maximal inhibitory concentration (IC_{50}) was determined by nonlinear curve fitting using Prism (GraphPad Software).

RESULTS

Expression of OATP1B3 in Human Pancreas

Real-time PCR was performed to determine whether transporters of the OATP1B subfamily are expressed in human pancreas tissue, revealing expression of OATP1B3 in human pancreas (expression relative to liver 0.39), while OATP1B1 was not detectable. Figure 1*A* shows the post-PCR gel electrophoresis, which had been performed to visualize the PCR products and which supports the notion that OATP1B3 might be expressed; kidney was included as negative control. Even if we did not observe expression of OATP1B3 in human placenta as previously reported (6), protein expression of the transporter in healthy pancreatic tissue was verified by Western blot analysis (Fig. 1*B*). Subsequently, localization of OATP1B3 in human pancreas was determined performing immunofluorescent staining, showing a specific fluorescent signal in the Langerhans islets. Similar results were obtained staining pancreas tissue slides using an anti-glucagon or an anti-insulin antibody. The comparison with the staining pattern of insulin suggested expression of OATP1B3 in the insulin-producing and -secreting β-cells rather than glucagon-producing α-cells. The herein-used pancreatic paraffin-embedded slides, protein, and mRNA sample were prepared of three different individuals.

Interaction of Sulfonylurea Derivatives With OATP1B3 Transport Function

The β-islet cells are the target of different insulinotropic antidiabetes drugs including the class of sulfonylurea derivatives and the class of glinids. In order to determine a potential function of OATP1B3 in pharmacokinetics of those compounds, we addressed the question of whether sulfonylurea derivatives and glinids interact with OATP1B3. A transporter overexpressing MDCKII cell line

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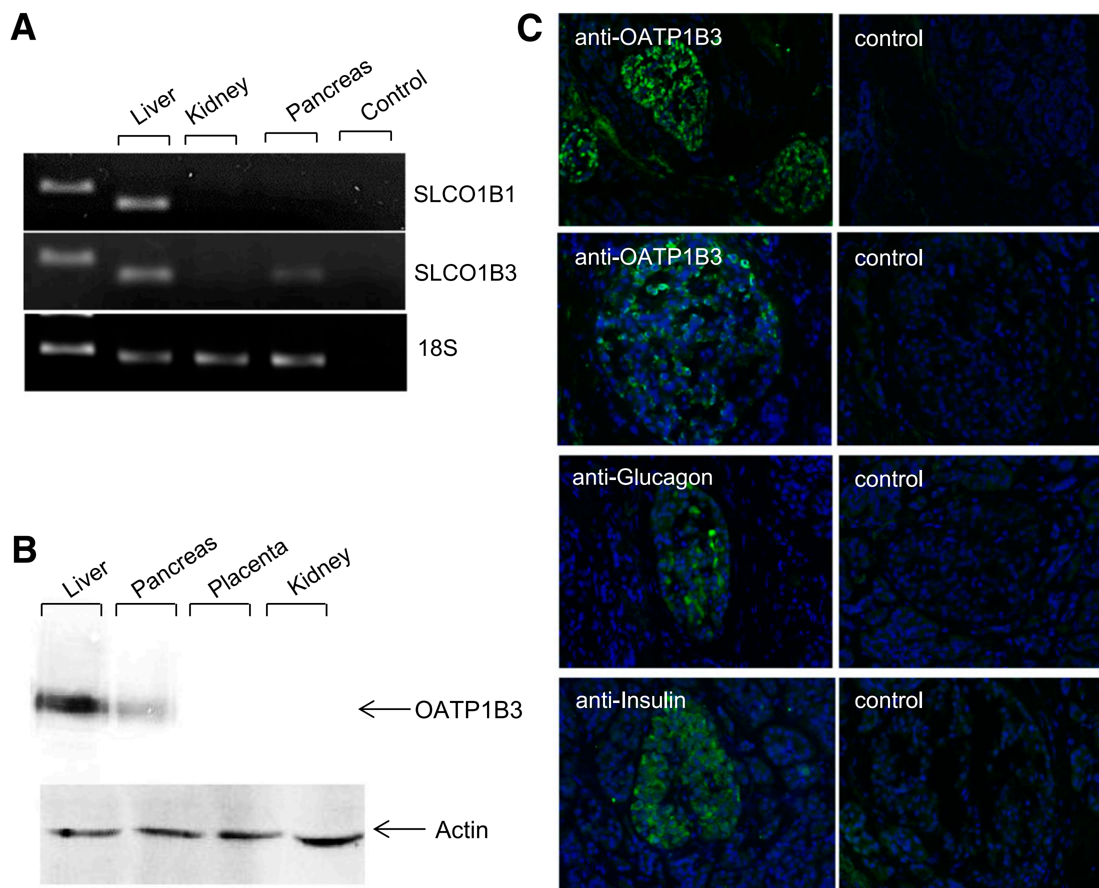

Figure 1—Expression and localization of OATP1B3 in human pancreas. At first, the expression of OATP1B transporters was determined performing real-time PCR (*A*) revealing transcripts of OATP1B3 in human pancreas. Protein expression of the transporter was verified by Western blot analysis using tissue lysates of liver, pancreas, kidney, and placenta (*B*). An enhanced fluorescent signal was observed in the islets of Langerhans by immunofluorescent staining of OATP1B3, insulin, and glucagon, respectively (*C*).

was generated and characterized for OATP1B3 expression and function (Fig. 2). Western blot analysis showed significant expression of OATP1B3, which was enhanced in the presence of 250 mmol/L butyrate. In addition, the cells exhibited significantly enhanced uptake of the previously reported OATP1B3 substrate CCK-8 sulfate (18,19). The maximum uptake rate (V_{max} ± SD) was 33.08 ± 3.3 nmol/μg protein/min, and the concentration at which half the maximum uptake occurs (K_m ± SD) was 25.06 ± 3.67 μmol/L. Concomitant exposure of tolbutamide, glibenclamide (also known as glyburide), glimepiride, and nateglinide with the OATP1B3 specific substrate [^{3}H]CCK-8 sulfate resulted in a significant inhibition of cellular accumulation of the radiolabeled substrate. The determined IC_{50} values were 10.0 μmol/L, 45.6 μmol/L, 29.9 μmol/L, and 6.5 μmol/L for tolbutamide, nateglinide, glimepiride, and glibenclamide, respectively (compare Fig. 3).

OATP1B3-Mediated Transport of Glibenclamide

The lowest IC_{50} was observed for the second-generation sulfonylurea derivative glibenclamide; therefore, this compound was selected to determine whether sulfonylurea derivatives are not only inhibitors, but are also substrates of OATP1B3. As shown in Fig. 4, OATP1B3-overexpressing cells exhibited time- and concentration-dependent accumulation of [^{3}H]glibenclamide. The apparent affinity (K_m) for glibenclamide was 33.32 ± 5.03 nmol/μg protein/min. The maximal velocity was 17.19 ± 0.85 μmol/L.

Impact of OATP1B3 on the Pharmacodynamic Effect of Glibenclamide in β-Islet Cells

It was the aim of subsequent experiments to determine whether expression of OATP1B3 has an impact on the pharmacodynamics of sulfonylurea derivatives in β-islet cells. MIN6 cells—a murine pancreatic β-cell line—were selected for those experiments, as these cells exhibit glucose-dependent insulin secretion (17). Importantly, MIN6 cells do express the sulfonylurea derivative–targeted ATP-sensitive K^+ channel (K_{ATP}) channel composed of the components SUR1/Kir6.2 (20,21). There was no expression of Oatp1b2 as determined by real-time PCR in the MIN6 cells (data not shown). First expression and secretion of insulin by MIN6 cells in the presence of low (2.8 mmol/L) and high (16.7 mmol/L) glucose were

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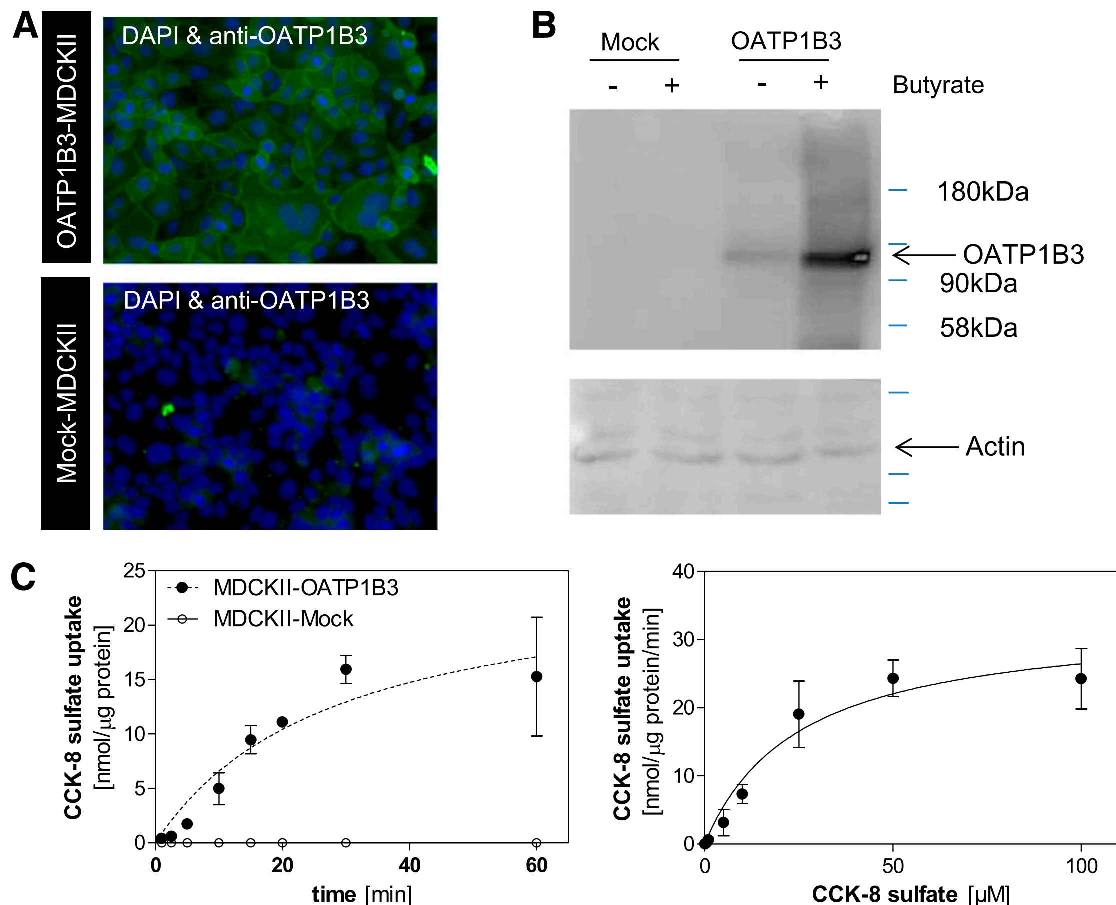

Figure 2—Characterization of an OATP1B3-overexpressing MDCKII cell line. Immunofluorescent staining using a previously reported specific OATP1B3 antibody showed expression of the transporter in the membrane of stably transfected MDCKII cells (*A*). OATP1B3 protein expression in MDCKII-vector control and MDCKII-OATP1B3 was determined by Western blot analysis before and after treatment with 250 μmol/L butyrate (*B*). Functionality of the expressed OATP1B3 was verified using the well-known substrate [^{3}H]CCK-8 sulfate (*C*).

determined revealing a nearly 15-fold increase of insulin as determined in the supernatant after treatment with high glucose for 2 h (insulin secretion fold-increase compared with 2.8 mmol/L glucose, mean ± SD 14.47 ± 0.40; Student *t* test, $P < 0.005$ [Supplementary Fig. 1]).

An adenoviral expression system was used to transiently express OATP1B3 in the MIN6 cells. Expression, localization, and function of OATP1B3 were verified in HeLa cells after adenoviral infection, as this cell line has previously been used for transport studies of OATP1B3 (22) (Supplementary Fig. 2). Infection with 25 plaque-forming units of the adenovirus (Ad-OATP1B3) resulted in cellular expression of the transporter in HeLa cells as determined by immunofluorescent staining. In addition, enhanced accumulation of the OATP1B3 substrates [^{3}H]CCK-8 sulfate, [^{3}H]bromosulfophthalein, and [^{3}H]-glibenclamide was observed (Supplementary Fig. 2). The kinetic parameters of adenoviral-expressed OATP1B3 determined for CCK-8 sulfate were comparable with those observed in MDCKII-OATP1B3 with a V_{max} of 37.1 ± 9.12 nmol/μg protein/min and a K_m of 19.89 ± 1.89 μmol/L. Adenoviral infection also resulted in significant expression of the transporter or the control protein eGFP in MIN6 cells as shown by Western blot analysis using infected or native MIN6 cell lysates (Fig. 5*A*). In addition, enhanced uptake of bromosulphthalein and glibenclamide was observed in the presence of increasing concentrations of the adenovirus pAD-OATP1B3 (Fig. 5*B*). The influence of OATP1B3 expression on glibenclamide-induced insulin secretion was assessed after adenoviral infection with Ad-OATP1B3 or Ad-EGFP as control. The presence of the uptake transporter significantly enhanced glibenclamide-induced insulin secretion (into the supernatant) of MIN6 cells. The impact of the transporter on glibenclamide-induced insulin secretion was dependent on the viral dose, as shown by experiments conducted in the presence of increasing concentrations of the virus (Supplementary Fig. 3). Importantly, presence of the transporter influenced neither glucose-induced insulin secretion (Fig. 5*C*) nor the expression of Ins1 or Ins2 in the cells (Fig. 5*D*).

DISCUSSION

Although transporters of the human OATP1B subfamily have often been described as liver specific (4,12), expression of OATP1B3 has previously been reported in

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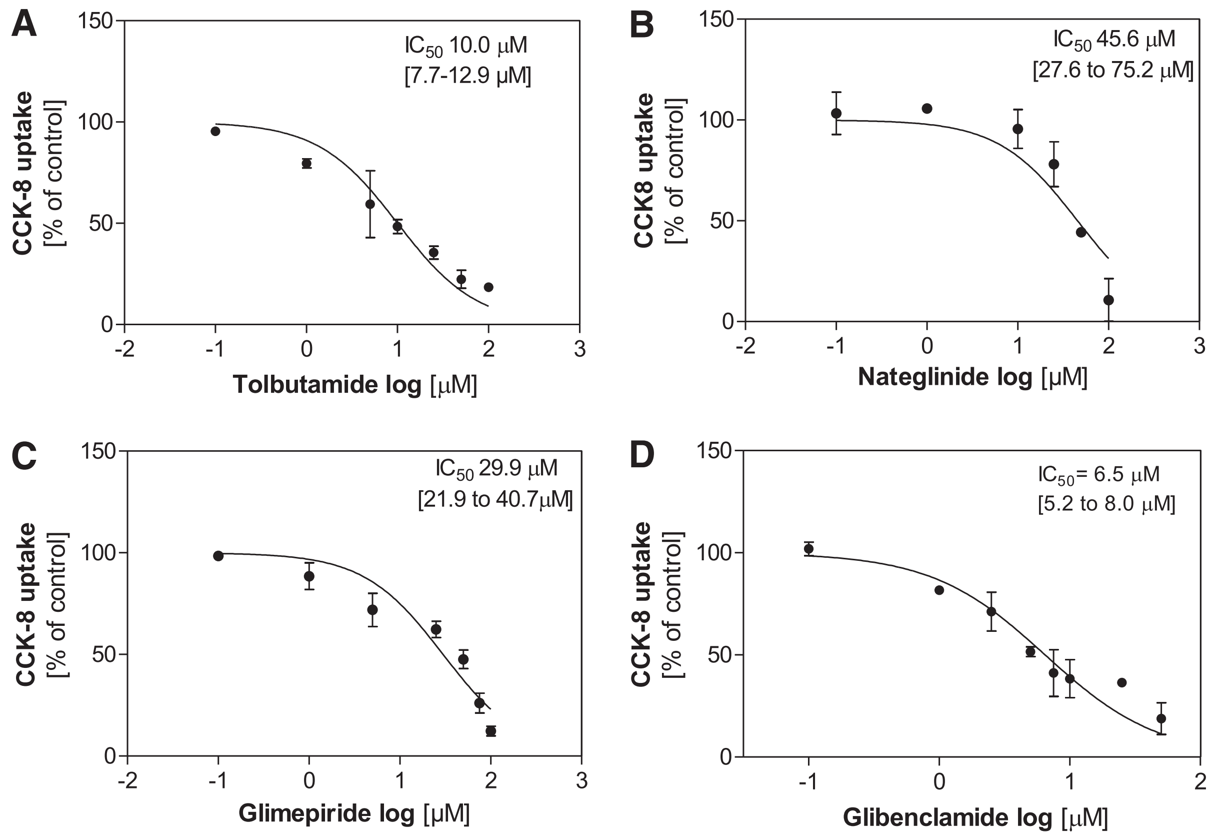

Figure 3—Inhibition of OATP1B3 in the presence of sulfonylurea derivatives and glinides. Uptake of sulfated [^{3}H]CCK-8 was determined in presence of increasing concentrations of tolbutamide (*A*), nateglinide (*B*), glimepiride (*C*), and glibenclamide (*D*). IC_{50} was estimated by nonlinear curve fitting using GraphPad Prism.

several extrahepatic tissues such as placenta (6), testis, retina (23), or solid cancers deriving from colon (24) or breast (25), thereby suggesting that this transporter is not restricted to human liver. Here, we report for the first time expression of OATP1B3 in the islets of Langerhans of human pancreas, likely localized within the insulin-secreting β-cells of the islets. Moreover, this study provides evidence that OATP1B3 enhances the insulinotropic effects of the sulfonylurea derivative glibenclamide, a newly identified substrate of OATP1B3.

Recent studies reported controversial findings regarding the presence of OATP1B3 in healthy pancreatic

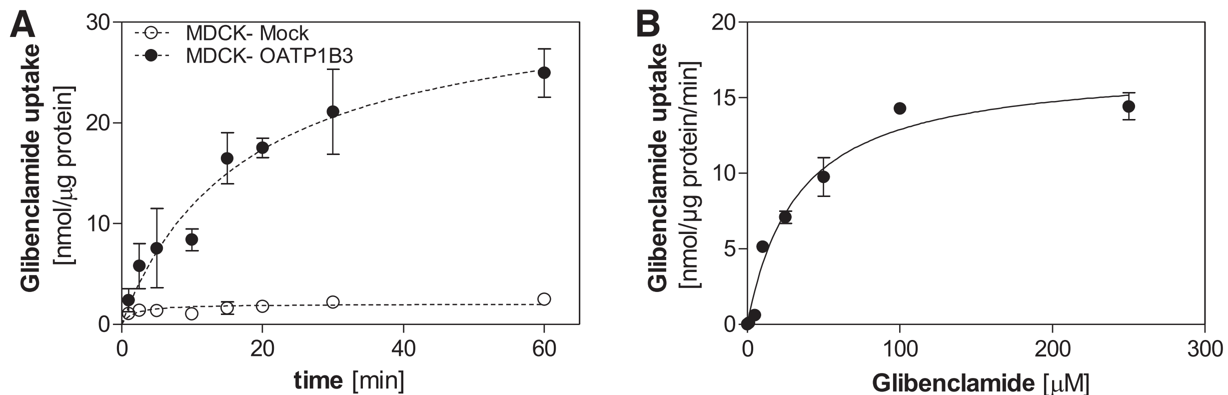

Figure 4—Transport of glibenclamide by OATP1B3. Time- (*A*) and concentration- (*B*) dependent uptake of [^{3}H]glibenclamide was determined in MDCKII cells stably transfected with OATP1B3. Parameters for saturation kinetics (V_{max} and K_m) were estimated by nonlinear curve fitting using GraphPad Prism.

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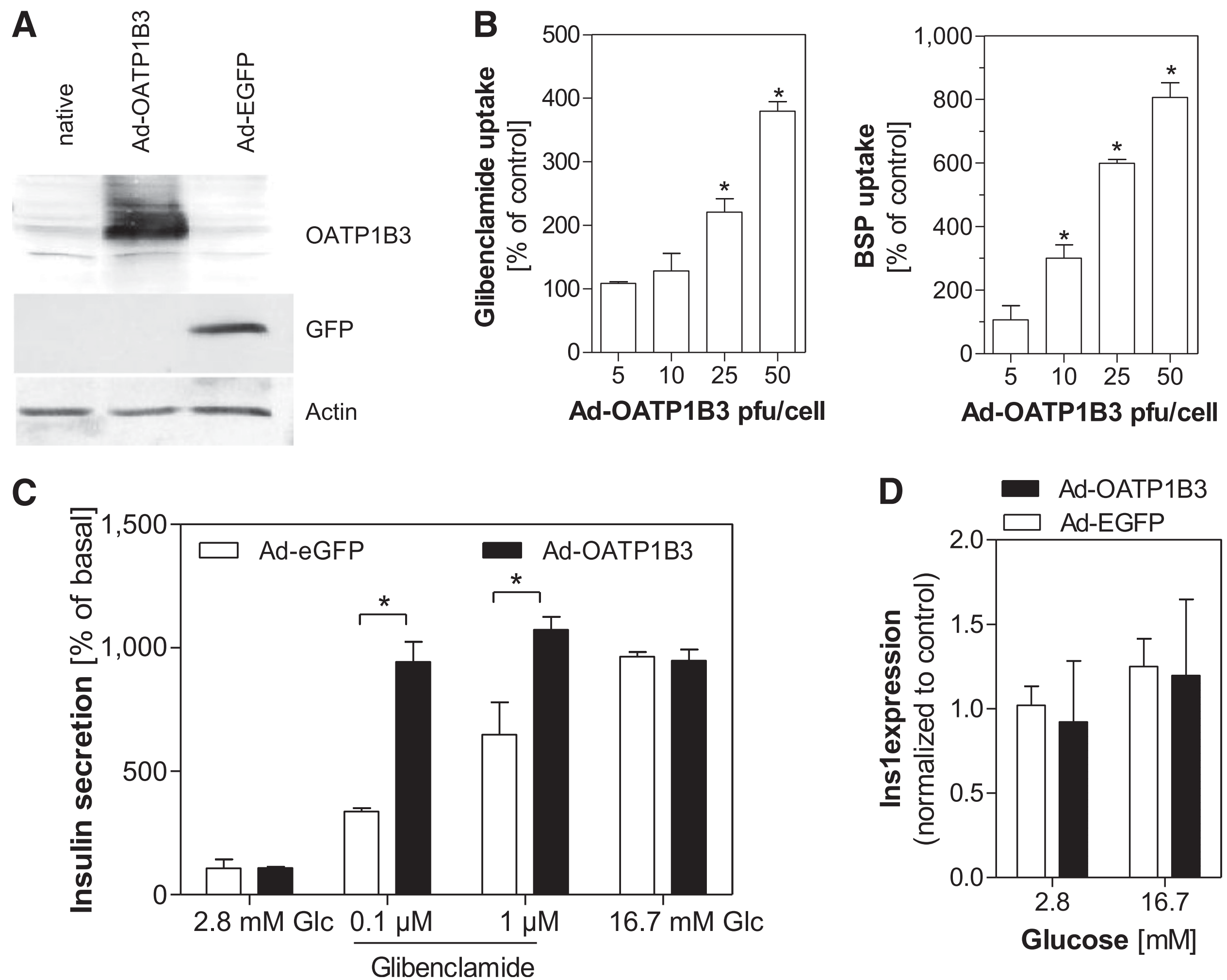

Figure 5—Impact of OATP1B3 on uptake and the insulinotropic effect of glibenclamide in β-islet cells. Protein expression of OATP1B3 and eGFP was assessed after adenoviral infection of MIN6 cells (*A*). The functionality of the transporter was determined after infection with increasing concentration of the adenovirus revealing enhanced uptake of glibenclamide or bromosulphthalein (BSP) in presence of OATP1B3 (*B*). After adenoviral infection with Ad-OATP1B3 or Ad-eGFP as control, MIN6 cells were treated with glibenclamide or glucose and the insulin concentration in the supernatant was determined by ELISA showing an enhanced insulinotropic effect of glibenclamide in presence of the transporter (*C*). Expression of Ins1 mRNA was determined after infection of MIN6 cells with Ad-OATP1B3 or Ad-eGFP as control (*D*). Data are expressed as mean ± SD. *$P < 0.05$, Student *t* test.

tissue (10,13,26). Thakkar et al. (26) identified a tumor-specific deletion variant of OATP1B3 exclusively expressed in malignant pancreatic tissue. However, the authors were not able to show expression of OATP1B3 wild type in normal pancreas by real-time PCR and immunohistochemistry using antibodies binding to the modified N-terminus or the COOH terminus of the protein. In accordance with our findings, OATP1B3 mRNA and protein have been detected in healthy human pancreas by others (10,13); however, the specific cellular localization had not been elucidated.

The β-islet cells of the islet of Langerhans are targets of insulinotropic antidiabetic medications such as sulfonylurea derivatives and glinids. The molecular target of sulfonylurea derivatives is the sulfonylurea receptor 1 subunit of the K_{ATP} channel (27). In order to provide evidence that pancreatic OATP1B3 may play a role in the tissue distribution and therefore insulinotropic effect of oral antidiabetes agents in the cellular microcompartment of β-islet cells, we first show that the second-generation sulfonylureas glibenclamide and glimepride, but also nateglinide, were able to inhibit OATP1B3-mediated CCK-8 uptake using OATP1B3-overexpressing cell lines. Previous studies mostly focused on the interaction of oral antidiabetes agents with OATP1B1. Interestingly, Bachmakov et al. (15) demonstrated inhibition of OATP1B1 and OATP1B3 transport by repaglinide in vitro, with IC_{50} values of 2.2 µmol/L and 5.6 µmol/L, respectively. The suggested role of OATP1B1 in hepatic uptake and therefore plasma exposure of repaglinide is in line with recent findings where healthy volunteers harboring the impaired function genotype SLCO1B1 521CC

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exhibited significantly higher plasma levels of repaglinide compared with wild-type carriers (summarized in 29). One may speculate that increased plasma concentration results in higher pancreatic exposure of the insulinotropic agent reflected in the observed trend for lower blood glucose levels in carriers of the mutant C allele. Similarly, nateglinide has been identified as an OATP1B inhibitor (14), and increased nateglinide plasma exposure was observed in individuals harboring two variant SLCO1B1 521 C alleles, suggesting a role of OATP1B1-mediated hepatic uptake for nateglinide (29). Conversely, there are also reports that do not confirm a role of OATP1B1 in the pharmacokinetics of nateglinide (30).

Controversial findings have been reported regarding the inhibition of OATP1B3-mediated uptake by glibenclamide in vitro ranging from no inhibition (31) to significant inhibition with IC_{50} values of 2.69 ± 0.12 μmol/L (16) and 9.8 ± 1.6 μmol/L (14). Our studies characterize glibenclamide as a substrate of OATP1B3 and confirm this sulfonylurea derivative as a competitive inhibitor of OATP1B3-mediated CCK-8 uptake. In comparison, several oral antidiabetes agents including glibenclamide were also reported to be potent inhibitors of OATP1B1-mediated transport of rosuvastatin, a well-known substrate of this transporter, and decreased hepatic uptake due to these medications has been predicted to result in increased statin plasma concentration (32). This is in accordance with findings reporting a significant role of OATP1B1 in pharmacokinetics of rosuvastatin (5). Hirano et al. (31) reported inhibition of OATP1B1-mediated uptake of pitavastatin; however, based on their prediction (calculation of the *R* value) the inhibition of OATP1B1 by glibenclamide seems of less clinical importance in terms of pharmacokinetics.

Previous studies assessing OATP1B transporters and their effects on the pharmacokinetics and -dynamics of substrate drugs have often focused on OATP1B1. Even if several functional genetic variants of OATP1B3 have been identified (33–35), the role of this transporter in vivo is rather unknown. This may be explained by the fact that the genetic variants in SLCO1B3 with severely reduced transport function are rare, and common nonsynonymous variants show only moderate functional impairment in vitro (22,37). In addition, there is broad substrate overlap between OATP1B3 and OATP1B1, with the latter exhibiting higher affinity to most of the thus far identified substrate drugs in clinical use (38). Accordingly, many pharmacogenetic studies on OATP1B3 with shared substrates failed to show an impact on pharmacokinetics. It seems noteworthy at this point, in contrast to OATP1B1, no significant impact of known genetic variants on the hepatic expression of OATP1B3 was observed (38). One of the compounds that seem to be transported exclusively by OATP1B3 is the chemotherapeutic agent paclitaxel. However, testing the influence of genetic variants on paclitaxel pharmacokinetics did not reveal any relevance in pharmacokinetics (39). Another OATP1B3-specific substrate is mycophenolic acid. In the case of this immunosuppressant, genetic variants of OATP1B3 have been associated with changes in drug disposition in vivo (40–41). Pharmacogenetic studies are an important tool to understand the function of a transporter in humans. However, as discussed above, studies on the impact of genetic variants of OATP1B3 have thus far contributed little to the understanding of the function of this transporter.

In this study, we show that adenoviral expression of OATP1B3 in MIN6 cells, an immortalized murine β-cell line, which exhibits sulfonylurea derivative and glinide-sensitive insulin secretion (42,43), results in a significantly enhanced pharmacodynamic activity of the OATP1B3 substrate glibenclamide. Sulfonylurea derivatives bind intracellularly to the SUR1 subunit of the K_{ATP} channel, resulting in the closure of the channel with cellular accumulation of potassium and subsequent depolarization of the β-cell membrane (21). The following opening of voltage-gated calcium channels stimulates the release of insulin from secretory granules (27). Previous findings suggest that glibenclamide in addition enhances protein kinase C–dependent exocytosis of insulin by inhibition of mitochondrial oxidation (44). Taken together, it is most likely that OATP1B3 could be an important determinant of the pharmacological efficacy of sulfonylurea derivatives, as it modulates entry of this class of drugs.

In conclusion, we report expression of OATP1B3 in the islets of Langerhans of the human pancreas. In addition, we showed that OATP1B3 facilitates cellular entry of glibenclamide, which results in enhanced sulfonylurea-induced insulin secretion in the presence of the human OATP1B3. It will be the aim of future studies to determine whether inhibition of OATP1B3, mediated either by drug-drug interactions or by genetic variants, influences pharmacodynamics of sulfonylurea derivatives or glinids.

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