The multidrug resistance proteins MRP2 (ABCC2) and MRP3 (ABCC3) are key primary active transporters involved in anionic conjugate and drug extrusion from the human liver. The major physiological role of MRP2 is to transport conjugated metabolites into the bile canaliculus, whereas MRP3 is localized in the basolateral membrane of the hepatocytes and transports similar metabolites back to the bloodstream. Both proteins were shown to interact with a large variety of transported substrates, and earlier studies suggested that MRPs may work as co-transporters for different molecules. In the present study we expressed the human MRP2 and MRP3 proteins in insect cells and examined their transport and ATPase characteristics in isolated, inside-out membrane vesicles. We found that the primary active transport of estradiol-17β-D-glucuronide (E217G), a major product of human steroid metabolism, was differentially modulated by bile acids and organic anions in the case of human MRP2 and MRP3. Active E217G transport by MRP2 was significantly stimulated by the organic anions indomethacin, furosemide, and probenecid and by several conjugated bile acids. In contrast, all of these agents inhibited E217G transport by MRP3. We found that in the case of MRP2, ATP-dependent vesicular bile acid transport was increased by E217G, and the results indicated an allosteric cross-stimulation, probably a co-transport of bile acids and glucuronate conjugates through this protein. There was no such stimulation of bile acid transport by MRP3. In conclusion, the different transport modulation of MRPs by bile acids and anionic drugs could play a major role in regulating physiological and pathological metabolite fluxes in the human liver.

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The homologous multidrug resistance ABC transporter proteins MRP2 and MRP3 seem to be key players in the transport of organic anionic conjugated compounds in the liver and kidney (1–7). Unlike the selective “classical” transport proteins, multidrug transporters recognize and handle a wide range of substrates. The members of the MRP family are transporting hydrophobic anionic conjugates but may also extrude hydrophobic uncharged drugs. In this latter case drug transport by MRPs has been shown to be linked to the co-transport or allosteric effect of cellular reduced glutathione, GSH (2, 3, 6–12).

MRP2 in polarized cells is localized in the apical (luminal) membrane surface, predominantly in the canalicular membrane of hepatocytes but also in the apical membranes of kidney-proximal tubules (1–3, 6, 13). In contrast, MRP3 expression in polarized cells is restricted to the basolateral membrane (4). The lack of functional MRP2 causes the human disease Dubin-Johnson syndrome, which is associated with a large increase of conjugated bilirubin and other conjugated metabolites in the bloodstream. Several animal models are available for modeling this disease condition (14, 15), and there are known mutations/polymorphisms, reducing human MRP2 activity and leading to disorders of conjugate metabolism (16–18).

Liver cells synthesize primary bile acids from cholesterol and then conjugate these compounds predominantly with taurine or glycine. The ABC transporter ABCB11 (also referred to as sister P-glycoprotein or bile salt export pump (BSEP)) is localized in the canalicular membrane and considered to be the major bile salt transporter (19, 20). However, MRP2 (7, 13, 21) and MRP3 (4, 5, 9) may also secrete these amphipathic compounds into the bile or the bloodstream, respectively. In the enterohepatic cycle a major part, about 95% of the secreted bile salts is reabsorbed in the intestine, whereas the rest is excreted into the feces after bacterial degradation. The relative role of the ABC transporters in this enterohepatic circulation is currently under study.

Elevated levels of MRP3 expression have been detected in human hepatocellular carcinoma (22) and in Dubin-Johnson patients, when in the absence of a functional MRP2, MRP3 seems to have a compensatory transport function (4, 7, 13). In this case several compounds, normally extruded into the bile, are transported by MRP3 into the sinusoidal blood. MRP3 is also up-regulated under cholestatic conditions and agents (23–25). Thus the co-regulated function of MRP2 and MRP3 may have a major effect on the conjugate metabolism and bile acid secretion in the human liver.

In the present paper we provide data for the interactions of MRP2 and MRP3 with estradiol-17β-D-glucuronide and bile acids, as well as with some pharmacologically important organic anions. A major metabolite of human estrogen metabolism, estradiol-17β-D-glucuronide (E217G), has been shown to be transported by both MRP2 (7, 13, 26, 27) and MRP3 (5, 28). This metabolite, with a significantly increased level during pregnancy and hormone replacement therapy, is secreted into the bile mainly by MRP2 (21, 29). Estrogen metabolites and
other steroid glucuronides show hepatotoxic effects (30) and mutually protect against cholestasis (31). In pregnancy and in hormone replacement therapy, taurocholate decreases E,17βG uptake in isolated rat hepatocytes (32), and certain MRP3 substrates induce MRP3 overexpression in cholestatic conditions (23–25). All of these data suggest an interrelated transport of bile acids and glucuronide-conjugated metabolites in the liver cells.

To explore these relationships we examined the transport properties of human MRP2 and MRP3, expressed at similar high levels in Sf9 cells. In isolated Sf9 membrane vesicles we measured both human MRP2- and MRP3-dependent direct vesicular uptake of labeled compounds, as well as the effects of these compounds on the MRP ATPase activity. Our data suggest that both MRP2 and MRP3 play important physiological roles in the transport of glucuronide conjugates and bile salts and that MRP2 performs a co-transport of glucuronide conjugates and bile salts into the bile canaliculi. In contrast, glucuronide transport into the bloodstream by MRP3 is inhibited by bile salts. We also demonstrate a differential modulation of these transport pathways by pharmacologically active organic anions. These results may help to understand the molecular basis of the complex interactions of metabolite and drug transport in the human liver and intestine.

EXPERIMENTAL PROCEDURES

Materials—E,17βG, glycololate (GC), glycochenocholeoycholate (GCDC), taurocholate (TDC), and taurochenocholeoycholate (TCDC) were obtained from Sigma. Labeled [3H]E,17βG was obtained from PerkinElmer Life Sciences, and [14C]GC was from Moravek Biochemicals.

Expression of MRP3 in Insect Cells—Recombinant baculoviruses containing the MRP cDNAs were prepared as described in Refs. 33 and 34. Sf9 (Spodoptera frugiperda) cells were cultured and infected with a baculovirus as described in Ref. 35. MRP2 and MRP3 cDNAs were obtained from Prof. Piet Borst and inserted into a baculovirus vector as described in Ref. 36.

Membrane Preparation and Immunoblotting—Virus-infected Sf9 cells were harvested, their membranes were isolated and stored, and the membrane protein concentrations were determined as described in Ref. 37. Gel electrophoresis and immunoblot detection were performed, and protein-antibody interaction was determined using the enhanced chemiluminescence technique as described in Ref. 36.

Membrane ATPase Measurements—ATPase activity was measured basically as described in Ref. 37, by determining the liberation of inorganic phosphate from ATP with a colorimetric reaction. The incubation mixture contained 10 mm MgCl2, 40 mm MOPS-Tris (pH 7.0), 50 mm KC1, 5 mm dithiothreitol, 0.1 mm EGTA, 4 mm sodium azide, 1 mm ouabain, and 4 mm ATP. Membrane ATPase activity was measured for 60 min at 37 °C in the presence of 4 mm ATP (control points), plus or minus 1 mm sodium orthovanadate (difference of the two values means the vanadate-sensitive component), and various concentrations of additional compounds, as indicated in the figures.

Transport Assay in Isolated Inside-out Membrane Vesicles—The membrane vesicles were incubated in the presence of 4 mm ATP in a buffer containing 10 mm MgCl2, 40 mm MOPS-Tris (pH 7.0), and 50 mm KC1 at 37 °C (34). Aliquots of the membrane suspensions were added to excess cold transport buffer and then rapidly filtered through nitrocellulose membranes (pore size, 0.45 μm). After washing the filters with 10 ml of ice-cold washing buffer and then rapidly filtered through nitrocellulose membranes (pore size, 0.45 μm), the radioactivity associated with the filters was measured by liquid scintillation counting. ATP-dependent transport was calculated by subtracting the values obtained in the presence of AMP from those in the presence of ATP. The figures present mean values obtained in three independent experiments.

RESULTS

Expression of Human MRP1, MRP2, and MRP3 in Insect Cells—Fig. 1A shows a Coomassie-stained blot of the proteins of isolated membranes obtained from Sf9 cells and separated by SDS gel electrophoresis. The Sf9 cells were infected with the recombinant baculoviruses inducing human MRP1, MRP2, or MRP3 expression. As documented, all three MRPs were successfully expressed at high levels (with an apparent molecular mass of about 160 kDa) in the Sf9 insect cells. The comparable amount of the expression of the three different human MRPs (about 5–7% of the total membrane proteins) allowed the direct comparison of the transport activities of these proteins in the following experiments. Immunoblotting by specific antibodies clearly identified the respective human MRP proteins expressed (Fig. 1B).

In the heterologous Sf9 expression system these human proteins were produced in an underglycosylated form that has been demonstrated not to have any effect on their transport functions (10, 33–37). In the following experiments we used isolated membranes, forming inside-out membrane vesicles from these human MRP-expressing Sf9 cells.
Vesicular Transport of E217βG by Human MRPs—In the following experiments we studied the transport of E217βG, a typical glucuronide conjugate, which may be a physiologically relevant model substrate of these transporters. This compound has been indicated to be transported substrate for both MRP2 and MRP3 (5, 7, 13, 26–28). By using isolated, inverted SF9 membrane vesicles, we have directly examined the vesicular transport of E217βG by the three different MRPs and examined the modulation of this transport by bile acids, bile salt conjugates, and organic anions.

Fig. 2 documents the ATP-dependent uptake of radiolabeled E217βG in isolated SF9 cell membrane vesicles expressing human MRP3 (panel A) or MRP2 (panel B). E217βG concentration dependence is shown. Membrane vesicle preparations were incubated with different concentrations of E217βG, including the labeled compound, at 37 °C for 2 min (see “Experimental Procedures”). B, inset, concentration dependence of E217βG uptake below 100 μM E217βG concentration. The mean values ± S.E. are shown.

AMP (which was low in all experiments). Also, as a control, we used vesicles obtained from SF9 cells expressing β-galactosidase. In these latter vesicles ATP-dependent tracer uptake was negligible. In all of these experiments the linear phase of the tracer uptake was determined (2 min for E217βG), and this period was used for studying the concentration dependence of the uptake.

As documented in Fig. 2A, MgATP-energized E217βG uptake in human MRP3-containing membrane vesicles was a saturable function of the E217βG concentration, with a calculated maximum uptake rate of about 1.3 nmol/mg membrane protein/min, and an apparent K_{m} value of about 25–30 μM.

When we measured E217βG uptake in SF9 membrane vesicles containing comparable amounts of human MRP1, the con-
membrane ATPase activity at E217 concentrations. In contrast, in membranes containing comparable amounts of human MRP2, there was a large increase in the ATPase activity at E217 concentrations, reinforcing that the vanadate-sensitive ATPase reflects transport-associated ATP hydrolysis by MRP2. These membrane ATPase experiments support the conclusions obtained from direct E217G transport experiments, suggesting that MRP3 (and MRP1) is a higher affinity but a much lower capacity transporter for E217G than MRP2.

Modulation of the MRP3- and MRP2-dependent Vesicular Transport of E217G by Organic Anions and Bile Salts—In the following experiments we examined the effects of the organic anions, furosemide, probenecid, and indomethacin (1M) on the direct, vesicular uptake of labeled E217G in MRP3-containing (Fig. 4A) and MRP2-containing (Fig. 4B) membranes, respectively. These experiments were carried out at two fixed E217G concentrations (1 and 13 µM) for both MRP3 and MRP2, to study these modulatory effects at E217G concentrations below the respective K_{m} values. We expected that both the inhibitory or the possible allosteric stimulatory effects could be optimally studied under these conditions.

We found that in the case of MRP3, E217G transport was inhibited by all of the three organic anions. The approximate K_{m} values were 350 µM for furosemide, 400 µM for probenecid, and 60 µM for indomethacin. In the case of MRP3, a slight 20–25% stimulation of E217G uptake was observed by low concentrations (5–10 µM) of indomethacin. Fig. 4A shows E217G uptake data measured at 1 µM E217G concentration, but similar results were obtained at higher (13 µM) E217G concentrations as well. We have already described (38) that both indomethacin and furosemide significantly stimulate MRP3 ATPase activity; thus both of these anions are most probably transported substrates of MRP3. Still, their predominant effect on E217G uptake was inhibitory.

As shown in Fig. 4B, in the case of MRP2, the effects of these organic anions were entirely different; furosemide and probenecid, between a wide concentration range of 50–500 µM significantly stimulated the ATP-dependent E217G uptake by MRP2, and this stimulation reached about 150% of the transport rate measured without these organic anions. Moreover, indomethacin in concentrations between 50 and 100 µM induced a 6–6.5-fold stimulation of E217G transport activity by MRP2, and a 5-fold stimulation of this transport was still observed at 500 µM indomethacin. Fig. 4B shows the data measured at 13 µM E217G concentration, but similar results were obtained at lower (1 µM) E217G concentrations as well.

In the following experiments we have studied the effect various bile salts conjugates on the vesicular uptake of labeled E217G by MRP3 (Fig. 5A) and MRP2 (Fig. 5B), respectively.
We have examined the effects of GC, GCDC, TDC, and TCDC, all potential physiological intrahepatic bile salts in humans. Again, these experiments were carried out at two fixed E217G/H9252G concentrations (1 and 13 μM) for both MRP3 and MRP2.

As shown in Fig. 5A, in the case of MRP3, E217G transport (measured at 1 μM E217G) was inhibited by all bile salts examined. In the case of GC this inhibition was more pronounced at about 100 μM, whereas the other bile salt conjugates strongly inhibited E217G already at 10 μM concentrations. When E217G uptake was measured at higher (13 μM) E217G concentrations, all bile salts were inhibitory as well (data not shown).

Fig. 5B documents that in the case of MRP2, all bile salts examined significantly stimulated ATP-dependent E217G uptake (measured here at 1 μM E217G). This stimulatory effect increased up to 100 μM of bile salt concentrations and reached about 180–200% in the case of GC, TCDC, and GCDC, whereas TDC was somewhat less effective in this stimulation.

All of these data indicate that the ATP-dependent active E217βG uptake, carried out by MRP2, is allosterically modulated by various organic anions and bile salt conjugates. To better characterize these interactions, we performed detailed E217βG concentration dependence studies by examining the effects of IM and that of the most abundant physiological bile salt conjugate in humans, GC in MRP2-containing membrane vesicles. We examined fixed concentrations (100 μM) of IM and GC, respectively, at an E217βG concentration range (10–100 μM), in which an S-shaped concentration dependence of E217βG uptake was observed (Fig. 2B).

As shown in Fig. 6, both IM and GC significantly stimulated the rate of E217βG uptake in this whole E217βG concentration range. Moreover, in the case of IM, a significant change in the shape of the curve was observed; the stimulation of E217βG uptake was more pronounced at lower E217βG concentrations.

Vesicular Transport of Labeled Glycocholate by Human MRPs—In the following experiments, to clarify the relationship...
between the transport of E217βG and its modulation by bile salts, we have directly measured labeled GC uptake in membrane vesicles containing MRP3 and MRP2, respectively. Labeled GC uptake was measured in a GC concentration range of 20–500 μM, and the effect of various concentrations of E217βG was examined.

Fig. 7A shows the magnitude of ATP-dependent labeled GC uptake by MRP3- or MRP2-containing membrane vesicles (and the same transport in β-galactosidase-containing membrane vesicles, as control), and the effect of 100 μM E217βG on this active transport process. As shown, both MRP3 and MRP2 containing membranes show a well measurable and comparable rate of GC uptake, with a tendency of saturation at about 300 μM GC concentration. The addition of E217βG in the case of MRP3 produced a slight inhibition of the GC uptake (at lower GC concentration this inhibition, because of the technical limitations, was not studied in detail). However, in the case of MRP2, in the entire GC concentration range studied, the addition of E217βG significantly increased (between 100 and 300 μM of GC, approximately doubled) the rate of GC uptake.

Fig. 7B demonstrates the combined transport rates of E217βG and GC in MRP2-containing vesicles under the above described experimental conditions. As documented, the addition of 100 μM GC in the presence of 20 μM E217βG results in a synergistic increase in the total ATP-dependent and vanadate-sensitive vesicular substrate uptake, significantly exceeding an additive effect for these two compounds. These data, in combination with the respective cross-stimulation of the MRP2-dependent vesicular transport of GC and E217βG (Figs. 6 and 7A), indicate a co-transport for these molecules by MRP2.

**DISCUSSION**

The MRP subfamily of the ABC proteins contains several ATP-dependent active transporters, which have important physiological functions in various organs, predominantly in the liver (2–7, 13). In the present study we focused on the comparative investigation of the in vitro ATPase and transport properties of human MRP2 and MRP3, both key players in liver metabolite transport.
We expressed these proteins in Sf9 baculovirus expression system, because the heterologous expression in insect cells produces high and comparable expression levels of various human ABC proteins. These proteins are correctly folded and inserted into the membrane environment, although in an underglycosylated form, which has been shown not to alter the transport activity of many multidrug resistance proteins (33–37).

The present experiments demonstrated major differences in the transport properties and inhibitor sensitivities of human MRP2 and MRP3. As documented earlier (2, 3, 7, 10), MRP2 is an efficient transporter for glutathione conjugates, e.g. NEM-GS and LTC₄, whereas the transport of these compounds by MRP3 in the Sf9 vesicles was negligible (4, 5, 11, 38), and none of the MRP3 substrates examined here had any stimulatory effect in this regard (38). Membrane ATPase activity measurements revealed a similar substrate dependence; in the case of MRP2, vanadate-sensitive ATPase activity is significantly stimulated by GS conjugates (36), whereas we found no such stimulation in the case of MRP3 (38).

When we examined the vesicular uptake of a previously documented MRP2 and MRP3 substrate, E₂₁₇βG (5, 7, 13, 26–28), we found a well measurable transport of this compound by both of these human proteins. In these experiments MRP3 showed a significantly lower capacity but higher affinity for transporting E₂₁₇βG than MRP2. In the case of MRP3, the
approximate $K_m$ value for $E_{217\beta G}$ transport was 25–30 $\mu M$, and the $V_{\text{max}}$ was 1.3 nmol/mg membrane protein/min. In the case of MRP2, the $K_m$ value could not be exactly determined, because the concentration dependence of $E_{217\beta G}$ showed a sigmoidal curve (see below), and the maximum uptake rate could be reached only above 1 m $E_{217\beta G}$ concentration. However, the $V_{\text{max}}$ in the case of MRP2 was about 10 times greater than for MRP3, reaching 12 nmol/mg membrane protein/min.

In the membrane ATPase measurements we found a similar phenomenon; in the MRP3-containing isolated membranes the ATPase activity was stimulated by relatively low concentrations (10–100 $\mu M$) of $E_{217\beta G}$, but this stimulation was much smaller than that found in the case of MRP2-containing membrane preparations. Moreover, $E_{217\beta G}$ stimulation of MRP2 ATPase activity was continuously increasing up to 1 mM of $E_{217\beta G}$ concentrations.

These data are in contrast to the high affinity $E_{217\beta G}$ transport found in earlier studies (13, 26), using isolated membranes of cells expressing human or rat MRP2. However, our data correlate closely to the findings reported by Zelcer et al. (39) and may suggest that a relatively low MRP2 expression level in mammalian cell membranes and/or a complex modulation of MRP2 transport (see below) may have masked this phenomenon (for a more detailed discussion, see the accompanying article (39)).

In the following experiments we have studied the modulation of the $E_{217\beta G}$ transport in MRP2- and MRP3-containing membranes by organic anions, which have been shown to exert significantly different effects on different MRPs. It has been reported earlier that furosemide, an anionic diuretic, has no major effect on MRP1 but strongly stimulates both MRP2 (36) and MRP3 ATPase activities (38). Probenecid, an inhibitor of MRP1, and indomethacin, a nonsteroid anti-inflammatory agent, were both found to stimulate MRP2 (36), and MRP3 ATPase activities (38). Benzbromarone, a strong inhibitor of MRP1 and MRP2, and MK571, a leukotriene receptor antagonist inhibitor of MRP1, also stimulated MRP3 ATPase activity in low concentrations (38).

When studying the direct modulatory effects of the above anionic compounds on $E_{217\beta G}$ uptake in isolated MRP3-containing inside-out membrane vesicles, we found a relatively weak but consistent inhibition by furosemide and probenecid, whereas indomethacin exerted a slight stimulatory effect at low (10–20 $\mu M$) concentration and a significant inhibitory effect at higher concentrations (Fig. 4A).

In MRP2-containing membrane vesicles the effects of these organic anions on $E_{217\beta G}$ uptake were entirely different; furosemide and probenecid induced an ~1.5-fold activation, whereas indomethacin produced a 6-fold increase in $E_{217\beta G}$ uptake (Fig. 4B). Thus a strong allosteric activation of the conjugate transport was observed in the case of MRP2. In the accompanying article, Zelcer et al. report major stimulatory effects of various organic anions on MRP2-dependent $E_{217\beta G}$ uptake. In this study the most effective stimulating agent was sulfanitran, but a large variety of anionic agents had similar effects.

We found a major difference between the modulation of MRP3 and MRP2 transport activities by bile salts as well. Again, $E_{217\beta G}$ uptake in isolated MRP3-containing inside-out vesicles was inhibited by all of the bile salts examined (GC, GCDC, TDC, and TCDC), whereas $E_{217\beta G}$ uptake in MRP2-containing vesicles was significantly stimulated by all of these conjugated bile salts (GCDC being the most effective, reaching a 2.5-fold stimulation), in a physiologically relevant 10–100 $\mu M$ concentration range (Fig. 5).

Because the $E_{17\beta G}$ transport by MRP2 showed a sigmoidal substrate concentration dependence between 10 and 100 $\mu M$ (Fig. 2B), we examined the possible allosteric modulation of this transport at these low $E_{17\beta G}$ concentrations, by the addition of 100 $\mu M$ indomethacin and GC, respectively. As shown in Fig. 6, in this concentration range a major stimulation was observed by both agents, although with somewhat different kinetics.

The demonstration of a different modulation of $E_{217\beta G}$ transport by MRP2 and MRP3 and the allosteric effects of several compounds in the case of MRP2 prompted us to directly examine the modulation of labeled GC uptake in isolated membrane vesicles. We could demonstrate an active, concentration-dependent, saturable GC uptake by both MRP2 and MRP3, with similar $V_{\text{max}}$ (100 and 250 pmol/mg membrane protein/min, respectively) and $K_m$ (150–200 $\mu M$) values. However, although GC uptake by MRP3 was slightly inhibited by the addition of 100 $\mu M$ $E_{217\beta G}$, in the case of MRP2 an about 2-fold activation of GC uptake was observed by 100 $\mu M$ $E_{217\beta G}$ (Fig. 7).

The relative contribution of MRP2 to the GC extrusion in hepatocytes is difficult to estimate, because the key bile acid transporter in the human liver is most probably BSEP (ABC11) (19, 20). The recent work by Noe et al. (20) suggests that BSEP has a high capacity and a high affinity (in the 5–15 $\mu$mol range) for various bile acids. Still, the co-stimulation of $E_{217\beta G}$ and GC transport in MRP2 may allow this transporter to become a significant contributor to bile salt transport under certain metabolic conditions.

The well measurable cross-stimulation of active $E_{217\beta G}$ and glycolic acid transport in the case of MRP2 demonstrates a positive allosteric modulation and the presence of at least two substrate binding sites in this protein. This cross-stimulation suggests a co-transport activity of MRP2 for these compounds, reinforced by the finding that the combined transport rate for the glucuronide-conjugate $E_{17\beta G}$ and the bile acid GC is significantly greater than that expected from an additive effect of these two substrates (Fig. 7B).

Our data indicate that under physiological conditions the secretion of bile salts and glucuronide conjugates by MRP2 is mutually facilitated by the accumulation of these compounds within the hepatocytes. Also, several anionic drugs, especially indomethacin, may greatly stimulate such a secretion process, yielding a better clearance for these cytotoxic metabolites. In contrast to MRP2, in the case of MRP3, only a cross-inhibition of the $E_{17\beta G}$ and glycolic acid transport could be observed, indicating a competition of these compounds on the transporter. This is not likely to be due to the absence of multiple binding sites in MRP3, as testified by the effects of some anionic compounds, which facilitate $E_{217\beta G}$ transport by MRP3 (38). The inhibitory (competitive) interaction of metabolites found here may have an important physiological consequence in preventing a rapid secretion of conjugates and bile acids back into the bloodstream.

Collectively, our results indicate that despite the overlapping specificities of MRP2 and MRP3, the different transport capacities and affinities for their common substrates resulted in significant differences for their actual transport properties. Our data suggest that MRP2 plays an important role not only in glutathione and glucuronide conjugates but also in bile salt conjugate extrusion in the liver. Moreover, the different modulatory effects of transported substrates may result in a transcellular cross-talk, involving MRP2 and MRP3, which reside in opposite membrane compartments.

In the case of MRP2, a high $V_{\text{max}}$, a relatively high $K_m$ (low affinity), and a cross-stimulation of the transported substrates with a simultaneous increase in substrate affinity assure an
efficient, rapidly adaptable function of this protein. Increases of cellular conjugated metabolite levels are properly handled by this large capacity, low affinity pump system, actively extruding these metabolites into the bile. In contrast, in the case of MRP3, a low \( V_{\text{max}} \) and a high \( K_m \) (higher affinity), and a competitive cross-inhibition of substrates result in a relatively low level function of this protein under normal conditions, extruding only a limited amount of selected metabolites into venous blood. The different expression levels of these two transport proteins in the respective liver cell membranes may also contribute to the physiological direction of metabolite transport. However, under long term stress conditions, e.g. during cholestasis, MRP3-dependent conjugate export provides an important rescue mechanism for the hepatocytes, based on the relatively slow process of up-regulation of MRP3 expression (23–25).

The present results, describing the substrate interactions of MRP2 and MRP3, may facilitate the understanding of conjugated metabolite transport in hepatocytes. They may also support the design and application of new agents modulating the function of MRP2 and MRP3 in transport-dependent metabolic processes.

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