Pergamon

Biochemical Pharmacology, Vol. 47, No. 12, pp. 2193–2200, 1994.

0006-2952/94 $7.00 + 0.00

0006-2952(94)E0084-X

CHARACTERIZATION OF TRANSPORT IN ISOLATED HUMAN HEPATOCYTES

A STUDY WITH THE BILE ACID TAUROCHOLIC ACID, THE UNCHARGED OUABAIN AND THE ORGANIC CATIONS VECURONIUM AND ROCURONIUM

GERRIE W. SANDKER, BETTY WEERT, PETER OLINGA, HENKE WOLTERS,*
MAARTEN J. H. SLOOFF,† DIRK K. F. MEIJER and GERRY M. M. GROOTHUIS‡

Departments of Pharmacology and Therapeutics, Pediatrics* and Surgery†, University Centre for Pharmacy, University of Groningen, Anthonius Deusinglaan 2, 9713 AW Groningen, The Netherlands

(*Received* 22 *July* 1993; *accepted* 9 *February* 1994)

Abstract—The uptake and efflux of three categories of substrates were measured in isolated human hepatocytes and compared to those in rat hepatocytes. In addition, the extent to which the *in vitro* experiments quantitatively reflect liver function *in vivo* in both species was investigated. The anionic bile acid taurocholic acid was taken up by isolated human hepatocytes at a considerably lower rate than observed in isolated rat hepatocytes. Taurocholic acid uptake both in human hepatocytes and in liver plasma membrane vesicles showed sodium dependency. The uptake rate of taurocholic acid in isolated hepatocytes of both species was quantitatively compatible with the reported liver clearance of the bile acid *in vivo*. Ouabain uptake rate in isolated human hepatocytes was lower than in rat hepatocytes. This species difference was in accordance with pharmacokinetic studies *in vivo* on hepatic clearance of ouabain in man and rat. Uptake of vecuronium into human hepatocytes was about a factor of 10 lower than that in rat hepatocytes. Uptake into and efflux from human hepatocytes was comparable for the two short acting muscle relaxants vecuronium and rocuronium. Since distribution to the liver is considered to be a major factor in termination of action of vecuronium and rocuronium these observations were in line with the human pharmacokinetic profiles. In conclusion, the uptake rate of the studied model compounds in human hepatocytes appeared to be lower than that in rat hepatocytes. These observed transport rates reflected the relative hepatic transport rates observed in these species in the intact organism, but the absolute values in both species for some substrates may have been somewhat lower than calculated from *in vivo* data. It is concluded that transport studies in isolated hepatocytes are suitable for comparative drug transport studies, but are less precise in the prediction of quantitative membrane transport.

Key words: human hepatocytes; drug transport; *in vitro–in vivo* correlation; interspecies differences; plasma membrane vesicles; sodium dependency

One of the functions of the liver is the clearance of various endogenous and exogenous compounds. This clearance process is determined by the net hepatic uptake, metabolism and biliary excretion processes. To clarify the mechanisms involved in transport of these substrates, *in vitro* techniques, such as the isolated perfused liver, isolated hepatocytes and plasma membrane vesicles have been employed extensively [1–17]. However, studies on mechanisms of hepatic transport of drugs in humans were limited to *in vivo* studies [18–20]. Due to the expansion of liver transplantation programs, human liver tissue became increasingly available for scientific research. The recent development of the methodology for the isolation of hepatocytes from human liver [21, 22] opens up the possibility to study hepatic transport processes in more detail in humans as well. Recently, liver plasma membrane vesicles were prepared from human liver, offering a second technique to study membrane transport of drugs in man *in vitro* [23, 24].

‡ Corresponding author. Tel. (31) 50-633280/633272; FAX (31) 50-633311.

In this study the uptake and excretion of three categories of substrates in human liver were investigated, using isolated human hepatocytes, i.e. the anionic bile acid taurocholic acid, the uncharged cardiac glycoside ouabain and the cationic muscle relaxants vecuronium and rocuronium.

Various carrier systems which are supposed to be specific with respect to the charge of the substrates have been identified in the liver [1, 14]. Carrier-mediated transport has been observed for bile acids, for other organic anions such as BSP and bilirubin, for uncharged compounds and for organic cations. For these classes of drugs separate carriers have been postulated and for the organic cations at least two uptake systems have been described: the type I uptake system for relatively small monovalent organic cations and the type II system for mostly bivalent organic cations with bulky ring structures [1, 25]. However, many interactions during hepatic transport have been observed with substrates with different charges [1, 15, 26], and overlapping substrate specificity is likely. The various substrates chosen in this study have previously been employed

in transport studies in rat hepatocytes [7, 9, 12, 13, 27, 28] and this enabled comparison of drug transport characteristics in rat and human hepatocytes. In the present study two type II organic cations were investigated, because clear interspecies differences in pharmacokinetics were observed *in vivo*: in humans no differences in duration of action for these compounds were found [19, 29], whereas in experimental animals the duration of action of rocuronium was considerably shorter than that of vecuronium [30–32]. We were curious to know whether these species differences could also be observed in isolated hepatocyte preparations from rat and human liver.

MATERIALS AND METHODS

Chemicals. The following compounds were obtained from the indicated sources: collagenase type VIII from Sigma Chemical Company (St Louis, MO, U.S.A.); BSA* was purchased from Organon Teknika (Boxtel, The Netherlands) and [16 β-*N*-methyl-^{3}H]rocuronium, [16 β-*N*-methyl-^{3}H]vecuronium (sp. act. 9.9 Ci/mmol) and unlabelled rocuronium (Org 9426) and vecuronium were kind gifts from Organon International BV (Oss, The Netherlands). Taurocholic acid-[^{3}H(G)] (sp. act. 8.1 Ci/mmol) and ouabain [^{3}H(G)] (sp. act. 18 Ci/mmol) were from Du Pont NEN Research Products (Boston, U.S.A.); taurocholic acid from Fluka Chemie AG (Buchs, Switzerland); ouabain from Merck (Darmstadt, Germany); Percoll from Pharmacia AB (Uppsala, Sweden); Picofluor 30 scintillation fluid was from Packard Instrument Inc. (Downers Grove, IL, U.S.A.). All other chemicals were of analytical grade and were obtained from commercial sources.

Hepatocyte isolation. Human liver tissue was obtained from livers harvested from multiorgan donors. Consent from legal authorities and family was obtained for the explantation of organs for transplantation purposes. If the donor livers had to be reduced for either "reduced size" liver transplantation in children or split for the use of both lobes, the liver tissue left over after the bipartition procedures was used for the isolation of hepatocytes. This included in case of a single "reduced size" transplantation the remaining lobe together with the caudate lobe and in case of transplantation of both lobes the caudate lobe. Whole livers discarded for transplantation after harvesting were used for hepatocyte isolation as well. Livers were perfused with UW and stored in this buffer at 4° until the start of the isolation procedure up to 43 hr. Human hepatocytes were isolated as described [33, Sandker *et al.*, manuscript in preparation]; by perfusing pieces of liver with collagenase. After the perfusion cells were washed with KHB [containing 118 mM NaCl, 5 mM KCl, 1.1 mM $MgSO_4$, 2.5 mM $CaCl_2$, 1.2 mM KH_2PO_4, 25 mM $NaHCO_3$, 10 mM glucose, 10 mM HEPES and 1% (w/v) BSA, saturated with O_2/CO_2 (95:5), pH 7.42]. Non-viable cells were removed by Percoll density centrifugation and the viable cells were suspended in KHB. The viability was assessed by TB exclusion (final concentration 0.2%), electron microscopy and reduction of MTT by mitochondrial dehydrogenases. When necessary, the cells were preserved overnight in UW solution before use, which was shown not to affect the transport rates in the cells [33, 34].

Non-fasted male Wistar rats (about 300 g) were anaesthetized with sodium pentobarbital (60 mg/kg, i.p.). Hepatocytes were isolated by collagenase perfusion. The procedure was a modification of that of Berry and Friend [35] and described in detail previously [6].

Uptake experiments in isolated hepatocytes. Uptake experiments were performed with [^{3}H]taurocholic acid (in human and rat hepatocytes a range from 2.6 to 336 μM), [^{3}H]ouabain (in human and rat hepatocytes 100 μM, about 0.5 K_m for rat hepatocytes [13, 28], [^{3}H]vecuronium (in human hepatocytes 100 μM and in rat hepatocytes 15 μM) and [^{3}H]-rocuronium (in human hepatocytes 100 μM) at 37°, using a rapid filtration technique as described before for rat hepatocytes [34]. Because of the very low uptake rate of 15 μM vecuronium (15 μM = K_m in rat hepatocytes [7]) in human hepatocytes observed in pilot experiments a higher vecuronium concentration was chosen for human than for rat hepatocytes. For rocuronium the same concentration was used as for vecuronium. Uptake in human hepatocytes was measured essentially by the same method as used for rat hepatocytes. However, the human hepatocytes were incubated in KHB with 60% Percoll. Percoll was added to prevent sedimentation of the cells during the uptake experiments, since in case of the human cells slower shaking was necessary to retain cell viability. Pilot experiments using rat hepatocytes showed that addition of Percoll did not influence the uptake rat of the substrates in rat cells (data not shown). The cell concentration used was 1.5×10^6 cells/mL, and the cells were pre-incubated 30 min before addition of the substrates. Cell viability (TB) was assessed again at the end of the uptake experiments.

To detect whether the taurocholic acid uptake in human hepatocytes showed sodium dependency, cells were pre-incubated at a 10 times higher hepatocyte concentration (15×10^6 cells/mL) in KHB oxygenated with O_2, in which the $NaHCO_3$ was replaced by NaCl. After the normal pre-incubation time of 30 min, cell suspensions were diluted either with KHB with 60% Percoll containing 143 mM NaCl or with KHB with 60% Percoll containing 143 mM choline chloride instead of NaCl. The [^{3}H]taurocholic acid was added with this dilution buffer resulting in final concentrations in a range from 2.6 to 168 μM. In this way taurocholic acid uptake was determined in the presence of 143 mM Na^+ or a 10 times lower Na^+ concentration. This procedure was chosen since the use of 60% Percoll impaired a complete and rapid separation of medium and cells by centrifugation, which was necessary in

* Abbreviations: BSA, bovine serum albumin; KHB, modified Krebs–Henseleit buffer; TB, Trypan blue; MTT, 3(4,5-dimethyl-thiazoyl-2-yl) 2,5 diphenyltetrazolium bromide; UW, University of Wisconsin organ preservation solution.

order to remove all the Na^+ from the incubation buffer.

Calculation of transport parameters in isolated hepatocytes. The initial uptake rate (V_i) was calculated from the initial slope of the curve during the linear part of the uptake rate curves. Linear aspects of the curves were observed in the first 3 min for taurocholic acid in human hepatocytes, the first 2.5 min for taurocholic acid in rat cells, the first 5 min for ouabain and the first 4 min for the other substrates in both rat and human cells. After some time the net uptake declined due to efflux from the cells and usually the cellular content reached a plateau value reflecting a situation in which uptake and efflux rate were equal. A plateau value for taurocholate was reached 9–21 min after adding the substrate in rat hepatocytes and after 30–60 min in human hepatocytes. For vecuronium and rocuronium the plateau value was reached 30–60 min after adding the substrate in both human and rat hepatocytes. For ouabain no plateau values could be attained within the time constraint defined by the incubation procedure.

These V_i and plateau values were used for the calculation of the transport parameters: fractional rate constants for uptake (k_{in}) and efflux (k_{out}). k_{in} was calculated using the equation $V_i = Q_i \times k_{in}$, in which Q_i is the amount of taurocholic acid in the medium at $t = 0$. k_{in} was corrected for the slight variations in the actual cell concentrations and was normalized for 10^6 cells/mL. k_{out} was calculated from the assumption that at plateau value the uptake rate equaled the efflux rate: $Q_p \times k_{out} = Q_m \times k_{in}$, where Q_p is the amount in the cells at plateau and Q_m the amount in the medium at plateau, calculated as $Q_i - Q_p$.

V_{max} and K_m for taurocholic acid uptake were calculated from experiments with concentration ranges of 2.6–336 μM, using Hanes–Woolf plots (substrate concentration/V_i vs substrate concentration). Values for V_{max} and K_m were determined from the reciprocal of the slope and negative x-intercept, respectively.

Initial intrinsic clearance by the cells was calculated from the ratio of the initial uptake velocity and the substrate concentration. From these data, an intrinsic clearance (Cl_i) for the whole liver could be calculated assuming a number of 114×10^6 cells/g liver [8] and a liver weight of 1.5 kg and 10.5 g for human and rat, respectively. Using the equation (based on the well stirred liver perfusion model) $E = Cl_i/(Cl_i + Q_H)$ in which E is the extraction ratio and Q_H the plasma flow through the liver (750 mL/min in man and 13 mL/min in rat [2, 8]), the initial E for the liver *in vivo* could be calculated from the *in vitro* data (E_{calc}).

Sodium-dependency of taurocholic acid uptake in isolated human plasma membrane vesicles. Plasma membrane vesicles (a mixture of basolateral and canalicular vesicles) were isolated as described earlier [23]. For determination of the sodium-dependency of taurocholic acid uptake, vesicles isolated from a single liver were used. These vesicles were stored frozen in liquid nitrogen until use. Frozen vesicles were thawed in a waterbath at 37° and diluted to the desired protein concentration

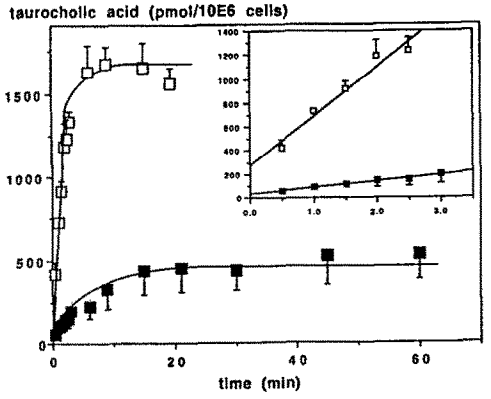

Fig. 1. Uptake of 10.5 μM [^{3}H]taurocholic acid into isolated human (closed symbols) and rat (open symbols) hepatocytes (N = 4). The inset shows the expanded data for the first 3 min. Values are expressed as means ± SEM.

(64 μg/20 μL) in a buffer with 300 mM sucrose, 10 mM HEPES, 10 mM $MgSO_4$ and 0.2 mM $CaCl_2$. [^{3}H]taurocholic acid uptake was measured using a rapid filtration technique. To 20 μL membrane vesicles, 80 μL Na-buffer (final concentration: 100 mM NaCl, 100 mM sucrose, 10 mM HEPES, 10 mM $MgSO_4$, 0.2 mM $CaCl_2$, BSA 1 mg/mL, pH 7.5) or K-buffer (containing 100 mM KCl instead of NaCl) with [^{3}H]taurocholic acid was added. Uptake was performed at 25°, with a final taurocholate concentration of 2.5 μM. Taurocholate uptake was stopped by adding 3 mL of cold wash buffer (4°, 100 mM KCl, 100 mM sucrose, 10 mM HEPES, 10 mM $MgSO_4$, 0.2 mM $CaCl_2$, pH 7.5) and filtration through cellulose nitrate filters, which had been previously rinsed with 1 mL 1 mM unlabelled taurocholic acid. Filters were washed twice with 3 mL of cold wash buffer and scintillation fluid was added for counting of radioactivity. Uptake values were corrected for the amount of [^{3}H]taurocholic acid at $t = 0$ (=binding to filter).

Statistics. Values are expressed as means ± SEM. Results were compared using the paired or unpaired Student's t-test. Differences were considered to be significant if $P < 0.05$.

RESULTS

The viability of the cell suspensions used in the

Table 1. Fractional rate constants for k_{in} and k_{out} of 10.5 μM [^{3}H]taurocholic acid in isolated human and rat hepatocytes

	Human	Rat
k_{in} (10^{-3}/min)	4.8 ± 2.0	38.2 ± 2.8*
k_{out} (10^{-3}/min)	89.3 ± 20.2	185.6 ± 12.5†

Results are expressed as means ± SEM (N = 4).
* $P < 0.001$ vs human.
† $P < 0.01$ vs human.

Table 2. Transport characteristics of [^{3}H]taurocholic acid in isolated human and rat hepatocytes

	K_m (μM)	V_{max} (pmol/min, 10^6 cells)
Human	62 ± 20	300 ± 100
Rat	24 ± 2	1800 ± 100*

Results are expressed as means ± SEM, human N = 4, rat N = 3.
* $P < 0.05$ vs human.

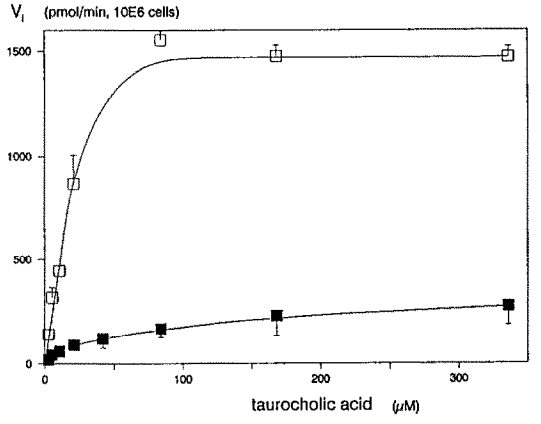

Fig. 2. V_i of [^{3}H]taurocholic acid expressed as pmol/min, 10^6 cells into isolated human (closed symbols) and rat (open symbols) hepatocytes as a function of the taurocholic acid concentration (N = 4). Values are expressed as means ± SEM.

present study was higher than 85% and 90%, respectively, for human and rat hepatocytes (as measured by TB exclusion just before pre-incubation was started). The viabilities measured with MTT confirmed the results obtained with TB. At the end of the uptake experiments, the viability of the cells was not decreased to a significant extend.

Time curves of uptake of 10.5 μM taurocholic acid in human and rat hepatocytes are shown in Fig. 1. Fractional rate constants for uptake and efflux, k_{in} and k_{out}, were calculated from these time curves and are shown in Table 1. Both the k_{in} and k_{out} for taurocholate were significantly higher in rat cells than in human cells.

In Fig. 2 the initial uptake rate of taurocholic acid at various concentrations ranging from 2.6 to 336 μM in human and rat cells is given. From these data the K_m and V_{max} were calculated. The K_m of taurocholate uptake was higher in isolated human hepatocytes than in rat hepatocytes, being 62 and 24 μM, respectively, but this difference was not statistically significant, at least not at a 5% level due to the rather large variation in the human cell studies (Table 2). The V_{max} was significantly lower in the human cells than in rat cells, 300 pmol/10^6 cells, min and 1800 pmol/10^6 cells, min respectively.

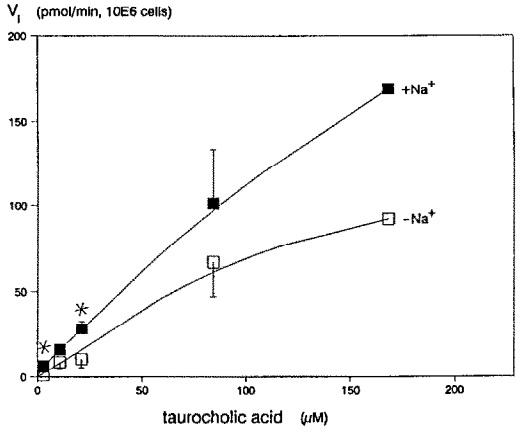

Fig. 3. Sodium-dependency of [^{3}H]taurocholic acid uptake in human hepatocytes. V_i of [^{3}H]taurocholic acid in a medium containing 143 mM NaCl (closed symbols) or in a medium containing 14.3 mM NaCl and 128.7 mM choline chloride (open symbols). 2.6 and 21 μM (N = 4), 10.5 μM (N = 5), 84 μM (N = 3) and 168 μM (N = 2). *$P < 0.05$ vs 14.3 mM Na^+. Values are expressed as means ± SEM.

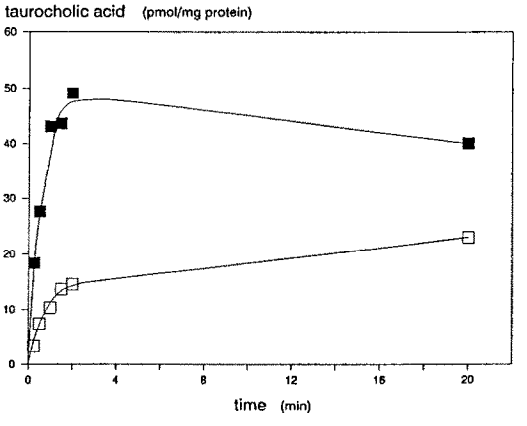

Fig. 4. Sodium dependency of taurocholic acid uptake into human liver plasma membrane vesicles. Uptake of 2.5 μM [^{3}H]taurocholic acid in plasma membrane fractions isolated from a single liver. Extravesicular medium containing 100 mM NaCl (closed symbols) or 100 mM KCl (open symbols).

The sodium dependency of [^{3}H]taurocholic acid uptake in isolated human hepatocytes is shown in Fig. 3. At all concentrations tested taurocholic acid uptake in a buffer with 14.3 mM Na^+ was lower than at 143 mM Na^+. This difference reached statistical significance (at $P < 0.05$) for the 2.6 and 21 μM [^{3}H]taurocholic acid incubations, using a paired Student's *t*-test. The difference was borderline significant for 10.5 μM ($P = 0.053$) but did not reach statistical significance for 84 (N = 3) and 168 μM (N = 2). Sodium dependency of taurocholic acid uptake was also determined in isolated human plasma membrane vesicles (Fig. 4). At the [^{3}H]-taurocholic acid concentration used (2.5 μM), the

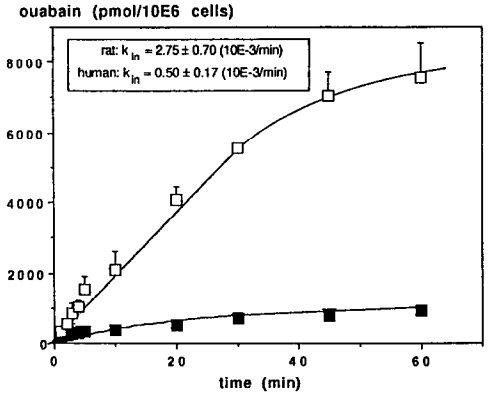

Fig. 5. Uptake of 100 μM [^{3}H]ouabain into isolated human (closed symbols) and rat (open symbols) hepatocytes. The values are expressed as means ± SEM. (In the curve of the human cells the SEM bars are smaller than the symbols). k_{in} values given in the insert are significantly different with $P < 0.005$ (N = 3).

uptake in the presence of an inwardly directed K^+-gradient was slower than in the presence of a Na^+-gradient.

In Fig. 5 the uptake of 100 μM ouabain in human and rat cells is shown. Fractional rate constants for uptake of [^{3}H]ouabain were lower in human than in rat hepatocytes, 0.50 ± 0.17 and 2.75 ± 0.70 × 10^{-3}/min ($P < 0.05$), respectively. No k_{out} values could be calculated, since no plateau value was reached during the incubation time used in the experiments.

The fractional rate constants for uptake and efflux of 100 μM vecuronium and rocuronium are depicted in Table 3. The uptake of 100 μM of the two muscle relaxants vecuronium and rocuronium was compared in hepatocytes isolated from the same three human donor livers. Transport of rocuronium into the cells seemed slightly faster than that of vecuronium (k_{in} was 1.4×10^{-3} and 0.79×10^{-3}/min, respectively, Table 3). However, this difference was not statistically significant at a 5% level (paired Student's t-test). Similarly, the k_{out} of rocuronium was not significantly different from the k_{out} of vecuronium (84×10^{-3} and 106×10^{-3}/min, respectively). Uptake of 15 μM [^{3}H]vecuronium into isolated rat hepatocytes was also measured (data not shown). The k_{in} and k_{out} observed for this transport were 6.9×10^{-3}/min and 33×10^{-3}/min, respectively.

Table 3. Fractional rate constants k_{in} and k_{out} of 100 μM [^{3}H]vecuronium and [^{3}H]rocuronium in isolated human hepatocytes

	Vecuronium	Rocuronium
k_{in} (10^{-3}/min)	0.79 ± 0.08	1.40 ± 0.33
k_{out} (10^{-3}/min)	106 ± 8	84 ± 14

Results are expressed as means ± SEM (N = 3).

Table 4. E_{calc} and $E_{in\ vivo}$ for taurocholic acid, ouabain and vecuronium

	Humans		Rat	
	E_{calc}	$E_{in\ vivo}$	E_{calc}	$E_{in\ vivo}$
Taurocholic acid	0.52	0.68 [18]	0.78	0.80 [40]
Ouabain	0.10	Unknown	0.20	0.82 [8]
Vecuronium	0.15	0.65 [20]	0.39	0.84 [32]

For taurocholic acid, ouabain and vecuronium the data on uptake in isolated hepatocytes were used to calculate E_{calc} for the whole liver, based on the assumptions described in the Materials and Methods. These data were compared to the available *in vivo* data obtained from the literature ($E_{in\ vivo}$), which are summarized in Table 4.

DISCUSSION

This study has shown that isolated human hepatocytes in suspension effectively take up organic substrates from the surrounding medium. However, in general, uptake into human hepatocytes appeared to be slower than uptake into isolated rat hepatocytes.

Taurocholic acid

In isolated rat hepatocytes the uptake of taurocholic acid was carrier-mediated and approximately 80% sodium dependent at a concentration of about 5–50 μM [11, 12, 36, 37]. At higher concentrations the relative involvement of the sodium-independent process increased [37]. V_{max} and K_m values found for total uptake of taurocholic acid in rat cells in the present study (1800 pmol/10^6 cells, min and 24 μM, respectively) were in good agreement with values detected in earlier studies. In these studies V_{max} varied from 1000 to 2800 pmol/10^6 cells, min and K_m varied between 18 and 39 μM [11, 12, 36, 38].

In human hepatocytes a two to three times higher K_m and a significantly lower V_{max} compared to rat hepatocytes were observed, indicating that taurocholic acid uptake in human hepatocytes was less efficient than in rat hepatocytes. This slower taurocholic acid uptake was also reflected in the significantly lower fractional rate constant for uptake (see Table 1), in accordance with the data of Kwekkeboom *et al.* [39]. The lower k_{out} in the human cells indicated that not only uptake into, but also taurocholate efflux from, the cells was slower than in rat hepatocytes.

Taurocholic acid uptake in the human liver appeared to be sodium-dependent, taking into account the observed differences in uptake rate at high and low Na^+ concentrations both in the hepatocytes (Fig. 3) and the isolated human liver plasma membrane vesicles (Fig. 4). In both preparations 75–80% of the initial taurocholic acid uptake appeared to be sodium-dependent at a concentration of 2.6 μM. This value agreed very well with the data obtained by Novak *et al.* [24]. The

smaller difference between uptake rate at high and low Na^+ concentrations at higher taurocholic acid concentrations may suggest that in human liver cells the relative involvement of the sodium-independent uptake process increases at higher concentrations.

In conclusion, taurocholic acid uptake in human hepatocytes exhibited sodium-dependency similar to that found in rat cells. However, the taurocholic acid transport at the uptake and efflux levels was slower in human than in rat hepatocytes.

In vivo, both in human and in rat, first pass liver extraction of taurocholic acid was high [18, 40]. To determine to what extent these *in vitro* experiments quantitatively reflected liver function *in vivo*, the initial extraction ratio for taurocholic acid was calculated from the parameters measured in isolated hepatocytes, these data are given in Table 4. Taking into account the various assumptions used for these calculations (number of cells/g liver, plasma flow, liver weight and well stirred liver), these *in vitro* values were in rather good agreement with the observed *in vivo* values.

Ouabain

Ouabain uptake mechanisms have been studied extensively in isolated rat hepatocytes [13, 28, 41–44]. The uptake process appeared to be carrier-mediated, energy dependent and sodium-independent. Evidence was found for independent transport systems for uptake of taurocholic acid and ouabain [42], but some overlap in substrate specificity was also suggested [13, 41, 44].

In the present study in isolated human hepatocytes a lower fractional rate constant for uptake of ouabain was observed than for rat cells. This is in line with the moderate hepatobiliary elimination of ouabain in humans (based on the scarcely available human *in vivo* data) compared to that in the rat [8]. The initial extraction ratios of the whole liver, calculated from the uptake of ouabain in isolated hepatocytes, were 0.20 and 0.10 for rat and humans, respectively. The initial extraction ratio found in the rat *in vivo* was considerably higher (Table 4). It should be realized however that for ouabain considerable distribution to other organs in the body takes place [8], which can at least partly be an explanation for the lower extraction ratio calculated from our *in vitro* data. Moreover, other possible explanations for the lower *in vitro* uptake rate can be that the membrane conformation changes during or after hepatocyte isolation which is not detected by the viability tests used, or alternatively that a selection of a subpopulation of hepatocytes is made during isolation [8]. Another possibility may be that diffusion of membrane carrier proteins over the cell surface alters the micro-environment of the particular protein molecules and thereby changes the affinity for the substrate [8, 45].

Vecuronium and rocuronium

In various animals, including the rat, the duration of action of rocuronium is considerably shorter than that of vecuronium, which can be explained by a higher liver clearance of rocuronium [30, 31]. According to our data and the data of Steen *et al.* [46] vecuronium is transported more slowly into isolated rat hepatocytes than rocuronium. Thus, the difference in duration of action of the two compounds, observed in the rat, seemed to be properly reflected in differences in uptake rate in isolated rat hepatocytes. Initial plasma clearance of both vecuronium and rocuronium appeared to be, to a large extent, due to liver uptake [19, 30, 47]. In humans no differences in duration of action were found [19, 29], indicating a similar uptake of both compounds. The similar transport parameters k_{in} and k_{out} for vecuronium and rocuronium in human hepatocytes (see Table 3) are in line with these *in vivo* data.

The k_{in} of vecuronium in isolated rat hepatocytes was a factor of 10 higher than in human hepatocytes (6.9×10^{-3}/min and 0.79×10^{-3}/min, respectively). In contrast, k_{out} was lower (33×10^{-3}/min and 106×10^{-3}, respectively) (Table 3 and [7]).

The values of the extraction ratio of vecuronium by human and rat liver calculated on the basis of the uptake in isolated hepatocytes, are lower than the values observed *in vivo*, as was also observed for ouabain and (as mentioned above) could at least partly be explained by initial extrahepatic distribution *in vivo*. Nevertheless, as is the case for ouabain and taurocholic acid, the species difference in kinetics of the cations as observed *in vivo* was clearly reflected in the isolated cells.

Conclusion

Interspecies differences between rat and humans reported in *in vivo* studies are reflected in the isolated hepatocytes. However, for both species a lower uptake rate into isolated hepatocytes than calculated from *in vivo* data may occur for some substrates. This can at least partly be explained by the influence of the considerable extrahepatic distribution on the initial plasma clearance *in vivo*. This implies that isolated hepatocytes can be used for comparative drug transport studies, but that they seem to be less suitable for quantitative drug transport studies.

Acknowledgements—Dr H. Proost is acknowledged for his help with calculation of the *in vivo* initial clearances, and Mrs W. K. Jonker for help in preparing the manuscript. Ir E. H. J. Oude Luttikhuis and Dr P. Olinga are thanked for preparing the figures. This study was performed in co-operation with the Human Liver Group Groningen and all members involved are gratefully acknowledged for their help. Part of this work, especially that concerning isolated human plasma membrane vesicles (Dr H. Wolters), was supported by grant 89–04 from the Netherlands Digestive Diseases Foundation.

REFERENCES

1. Meijer DKF, Transport and metabolism in the hepatobiliary system. In: *Handbook of Physiology. The Gastrointestinal System, Vol. III* (Eds. Schulz SG, Forte JG and Rauner BB), pp. 717–758. Oxford University Press, New York, 1989.
2. Meijer DKF and Groothuis GMM, Hepatic transport of drugs and proteins. In: *Oxford Textbook of Clinical Hepatology, Vol. 1* (Eds. McIntyre N, Benhamou J-P, Bircher J, Rizzetto M and Rodes J), pp. 40–78. Oxford University Press, Oxford, 1991.
3. Braakman I, Groothuis GMM and Meijer DKF, Acinar

redistribution and heterogeneity in transport of the organic cation rhodamine B in rat liver. *Hepatology* **7**: 849–855, 1987.
4. Groothuis GMM, Keulemans KPT, Hardonk MJ and Meijer DKF, Acinar heterogeneity in hepatic transport of dibromosulfophthalein and ouabain studied by autoradiography, normal and retrograde perfusions and computer simulation. *Biochem Pharmacol* **32**: 3069–3078, 1983.
5. Kroker R, Answer MS and Hegner DA, A compartmental model for hepatic transport of taurocholic acid in isolated perfused rat liver. *Naunyn-Schmiedebergs Arch Pharmacol* **303**: 287–293, 1978.
6. Braakman I, Pijning T, Verest O, Weert B, Meijer DKF and Groothuis GMM, Vesicular uptake system for the cation Lucigenin in the rat hepatocyte. *Mol Pharmacol* **36**: 537–542, 1989.
7. Mol WEM, Fokkema GN, Weert B and Meijer DKF, Mechanisms for the hepatic uptake of organic cations. Studies with the muscle relaxant Vecuronium in isolated rat hepatocytes. *J Pharmacol Exp Ther* **244**: 268–275, 1988.
8. Blom A, Scaf AHJ and Meijer DKF, Hepatic drug transport in the rat. A comparison between isolated hepatocytes, the isolated perfused liver and the liver in vivo. *Biochem Pharmacol* **31**: 1553–1565, 1982.
9. Scharschmidt BF and Stephens JE, Transport of sodium, chloride, and taurocholate by cultured rat hepatocytes. *Proc Natl Acad Sci USA* **78**: 986–990, 1981.
10. Steen H, Oosting R and Meijer DKF, Mechanisms for the uptake of cationic drugs by the liver: a study with tributylmethylammonium (TBuMA). *J Pharmacol Exp Ther* **258**: 537–543, 1991.
11. Minder E and Paumgartner G, Disparate Na^+-requirement of taurocholate and indocyanine green uptake by isolated hepatocytes. *Experientia* **35**: 888–890, 1979.
12. Schwarz LR, Burr R, Schwenk M, Pfaff E and Greim H, Uptake of taurocholic acid into isolated rat-liver cells. *Eur J Biochem* **55**: 617–623, 1975.
13. Schwenk M, Wiedman T and Remmer H, Uptake, accumulation and release of ouabain by isolated rat hepatocytes. *Naunyn-Schmiedebergs Arch Pharmacol* **316**: 340–344, 1981.
14. Schwenk M, Burr R, Schwarz LR and Pfaff E, Uptake of bromosulfophthalein by isolated liver cells. *Eur J Biochem* **64**: 189–197, 1976.
15. Zimmerli B, Valantinas J and Meier PJ, Multispecificity of Na^+-dependent taurocholate uptake in basolateral (sinusoidal) rat liver plasma membrane vesicles. *J Pharmacol Exp Ther* **250**: 301–308, 1989.
16. Meier PJ, Meier-Abt ASt, Barrett C and Boyer JL, Mechanisms of taurocholate transport in canalicular and basolateral rat liver plasma membrane vesicles. Evidence for an electrogenic canalicular organic anion carrier. *J Biol Chem* **259**: 10614–10622, 1984.
17. Nishida T, Gatmaitan Z, Che M and Arias IM, Rat liver canalicular membrane vesicles contain an ATP-dependent bile acid transport system. *Proc Natl Acad Sci USA* **88**: 6590–6594, 1991.
18. Cowen AE, Korman MG, Hofmann AF and Thomas PJ, Plasma disappearance of radioactivity after intravenous injection of labeled bile acids in man. *Gastroenterology* **68**: 1567–1573, 1975.
19. Wierda JMKH, Kleef UW, Lambalk LM, Kloppenburg WD and Agoston S, The pharmacodynamics and pharmacokinetics of Org 9426, a new non-depolarizing neuromuscular blocking agent, in patients anaesthetized with nitrous oxide, halothane and fentanyl. *Can J Anaesth* **38**: 430–435, 1991.
20. Sohn YJ, Bencini AF, Scaf AHJ, Kersten UW and Agoston S, Comparative pharmacokinetics and dynamics of vecuronium and pancuronium in anesthetized patients. *Anesth Analg* **65**: 233–239, 1986.
21. Houssin D, Capron M, Celier C, Cresteil T, Demaugre F and Beaune P, Evaluation of isolated human hepatocytes. *Life Sci* **33**: 1805–1809, 1983.
22. Tee LBG, Seddon T, Boobis AR and Davies DS, Drug metabolising activity of freshly isolated human hepatocytes. *Br J Clin Pharmacol* **19**: 279–294, 1985.
23. Wolters H, Spiering M, Gerding A, Slooff MJH, Kuipers F, Hardonk MJ and Vonk RJ, Isolation and characterization of canalicular and basolateral plasma membrane fractions from human liver. *Biochim Biophys Acta* **1069**: 61–69, 1991.
24. Novak DA, Ryckman FC and Suchy FJ, Taurocholate transport by basolateral plasma membrane vesicles isolated from human liver. *Hepatology* **10**: 447–453, 1989.
25. Meijer DKF, Mol WEM, Müller M and Kurz G, Carrier-mediated transport in the hepatic distribution and elimination of drugs, with special reference to the category of organic cations. *J Pharmacokinet Biopharm* **18**: 35–70, 1990.
26. Frimmer M, Organotropism by carrier-mediated transport. *Trends Pharmacol Sci* **2**: 395–397, 1982.
27. Schwarz LR and Watkins JB, Uptake of taurocholate, a vecuronium-like organic cation, Org 9426, and ouabain into carcinogen-induced diploid and polyploid hepatocytes obtained by centrifugal elutriation. *Biochem Pharmacol* **43**: 1195–1201, 1992.
28. Eaton DL and Klaassen CD, Carrier-mediated transport of ouabain in isolated hepatocytes. *J Pharmacol Exp Ther* **205**: 480–488, 1978.
29. Agoston S, Salt P, Newton D, Bencini A, Boomsma P and Erdmann W, The neuromuscular blocking action of Org NC 45, a new pancuronium derivative, in anaesthetized patients. A pilot study. *Br J Anaesth* **52**: 53S–59S, 1980.
30. Khuenl-Brady K, Castagnoli KP, Canfell PC, Caldwell JE, Agoston S and Miller RD, The neuromuscular blocking effects and pharmacokinetics of Org 9426 and Org 9616 in the cat. *Anesthesiology* **72**: 669–674, 1990.
31. Muir AW, Huston J, Marshall RJ, Bowman WC and Marshall IG, A comparison of the neuromuscular blocking and autonomic effects of two new short-acting vecuronium analogues with those of succinylcholine in the anaesthetized cat and pig. *Anesthesiology* **70**: 533–540, 1989.
32. Waser PG, Wiederkehr H, Chang Sin-Ren A and Kaiser-Schönenberger E, Distribution and kinetics of ^{14}C-vecuronium in rats and mice. *Br J Anaesth* **59**: 1044–1051, 1987.
33. Sandker GW, Weert B, Slooff MJH and Groothuis GMM, Preservation of isolated rat and human hepatocytes in UW solution. *Transplant Proc* **22**: 2204–2205, 1990.
34. Sandker GW, Slooff MJH and Groothuis GMM, Drug transport, viability and morphology of isolated rat hepatocytes preserved for 24 hours in University of Wisconsin solution. *Biochem Pharmacol* **43**: 1479–1485, 1992.
35. Berry MN and Friend DS, High-yield preparation of isolated rat parenchymal cells. A biochemical and fine structural study. *J Cell Biol* **43**: 506–520, 1969.
36. Hardison WGM, Bellentani S, Heasley V and Shellhamer D, Specificity of an Na^+-dependent taurocholate transport site in isolated rat hepatocytes. *Am J Physiol* **246**: G477–G483, 1984.
37. Anwer MS and Hegner D, Effect of Na^+ on bile acid uptake by isolated rat hepatocytes. Evidence for a heterogenous system. *Hoppe-Seyler's Z Physiol Chem* **359**: 181–192, 1978.
38. Blitzer BL, Ratoosh SL, Donovan CB and Boyer JL, Effect of inhibitors of Na^+-coupled ion transport on

bile acid uptake by isolated rat hepatocytes. *Am J Physiol* **243**: G48–G53, 1982.
39. Kwekkeboom J, Princen HMG, van Voorthuizen EM, Meijer P and Kempen HJM, Comparison of taurocholate accumulation in cultured hepatocytes of pig, rat and man. *Biochem Biophys Res Commun* **162**: 619–625, 1989.
40. Deroubaix X, Coche T, Depiereux E and Feytmans E, Saturation of hepatic transport of taurocholate in rats *in vivo*. *Am J Physiol* **260**: G189–G196, 1991.
41. Petzinger E, Fischer K and Fasold H, Role of the bile acid transport system in hepatocellular ouabain uptake. In: *Cardiac Glycosides 1785–1985. Biochemistry, Pharmacology, Clinical Relevance* (Eds. Erdmann E, Greeff K and Skou JC), pp. 297–304. Steinkopff-Verlag, Darmstadt, 1986.
42. Eaton DL and Richards JA, Kinetic evaluation of carrier-mediated transport of ouabain and taurocholic acid in isolated rat hepatocytes. Evidence for independent transport systems. *Biochem Pharmacol* **35**: 2721–2725, 1986.
43. Brown DJ and Hunter A, The effect of thioacetamide on sulfobromophthalein and ouabain transport in isolated rat hepatocytes. *Toxicology* **32**: 165–176, 1984.
44. Petzinger E and Fischer K, Transport functions of the liver. Lack of correlation between hepatocellular ouabain uptake and binding to (Na^+, K^+)-ATPase. *Biochim Biophys Acta* **815**: 334–340, 1985.
45. Groothuis GMM, Hulstaert CE, Kalicharan D and Hardonk MJ, Plasma membrane specialization and intracellular polarity of freshly isolated rat hepatocytes. *Eur J Cell Biol* **26**: 43–51, 1981.
46. Steen H, Merema MT and Meijer DKF, A multispecific uptake system for taurocholate, cardiac glycosides and cationic drugs in the liver. *Biochem Pharmacol* **44**: 2323–2331, 1992.
47. Bencini AF, Scaf AHJ, Sohn YJ, Kersten-Kleef UW and Agoston S, Hepatobiliary disposition of vecuronium bromide in man. *Br J Anaesth* **58**: 988–995, 1986.