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A MULTISPECIFIC UPTAKE SYSTEM FOR TAUROCHOLATE, CARDIAC GLYCOSIDES AND CATIONIC DRUGS IN THE LIVER

HERMAN STEEN,* MARJOLIJN MEREMA and DIRK K. F. MEIJER
Department of Pharmacology and Therapeutics, University Centre for Pharmacy, Groningen State University, Ant. Deusinglaan 2, 9713 AW Groningen, The Netherlands

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Abstract—To test the hypothesis of multiplicity in carrier-mediated uptake mechanisms for organic cations in the liver and to study the possible relation with bile acid and cardiac glycoside uptake mechanisms, mutual interaction during uptake of various radiolabeled quaternary amines has been studied in isolated rat hepatocytes. Inhibition patterns at low concentrations (1 μM) of the presumed type I monovalent organic cation tri-*n*-butylmethylammonium were markedly different from those at relatively high concentrations (25 μM). Both the cardiac glycoside K-strophantoside and the bile acid taurocholate clearly reduced the uptake rate of tri-*n*-butylmethylammonium at 25 μM whereas these agents completely failed to reduce the uptake at low concentrations of the cation. Subsequently, inhibition of uptake of some multivalent amphipathic organic cations (muscle relaxants) for the type II uptake system was investigated. It was found that the uptake of these muscle relaxants both at tracer concentrations (<1 μM) and at relatively high concentrations (25 μM) was decreased in the presence of low concentrations of the cardiac glycoside K-strophantoside, while taurocholate only inhibited the uptake at the concentration range >25 μM of the muscle relaxants. Procainamide ethobromide, a typical type I organic cation, did not affect the uptake either at the low or high concentration range of the muscle relaxants. It is concluded that for each of the type I-like compounds and type II-like compounds tested at least two systems are involved in uptake into hepatocytes: tri-*n*-butylmethylammonium in a concentration range $\leq$ 1 μM is mainly taken up by the type I uptake system and at concentrations $\geq$ 25 μM also by system(s) that can be inhibited by taurocholate and K-strophantoside. Bulky amphipathic organic (type II) cations at concentrations <1 μM are also transported by an uptake system that is inhibitable by cardiac glycosides but not by bile salts. At concentrations > 25 μM these compounds are predominantly accommodated by an uptake system that possibly mediates uptake of both cardiac glycosides and bile acids. This concept was supported by the observation that both type II organic cations and bile salts can inhibit ouabain uptake, while type II organic cations as well as the cardiac glycosides reduce taurocholate uptake rate. The present data support the idea that the liver seems to be equipped with a "multispecific" uptake system that transports hydrophobic compounds irrespective of charge, including some type I and type II organic cations at relatively high substrate concentrations.

It has been postulated earlier that uptake of cationic drugs by the liver occurs via at least two separate carrier-mediated pathways, as recently reviewed [1, 2]. Differences between these transport systems were observed with respect to their substrate specificity. Labeling of different proteins using photolabile derivatives of type I and type II compounds also indicates the presence of two different uptake systems [2, 3].

The monovalent organic cations procainamide ethobromide (PAEB†) [4–7], its azido derivative APM [8] and its *N*-acetyl derivative APAEB [6, 9, 10] are proposed to be transported by a "type I organic cation" uptake system. Larger, often multivalent, organic cations, in which the positive charge is integrated in bulky ring structures, are supposed to be transported by a "type II organic cation" uptake system. The major differences in substrate specificity observed between the two transport systems were thought to concern the interactions with cardiac glycosides and bile acids [1, 2]. The cardiac glycosides K-strophantoside and digitoxin showed a potent inhibiting effect on the transport of the type II compounds hexafluronium [11], *d*-tubocurarine [12] and vecuronium [13]. The bile acid taurocholate also showed an inhibiting effect on the hepatic uptake of the type II compounds *d*-tubocurarine [14] and vecuronium [13]. On the contrary, neither cardiac glycosides nor bile acids have any effect on the type I uptake system [8, 14, 15]. In addition, type II compounds can block the uptake of type I compounds, whereas type I compounds do not inhibit the uptake of type II compounds (even at high concentrations). It is not clear how cardiac glycosides and bile acids do interfere with the transport of the the type II cationic compounds.

* Corresponding author: Dr H. Steen, Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Department of Blood Coagulation, Plesmanlaan 125, 1066 CX Amsterdam, The Netherlands. Tel. (31) 20-5123574; FAX (31) 20-5123332.

† Abbreviations: TBuMA, tri-*n*-butylmethylammonium; PAEB, procainamide ethobromide; APAEB, *N*-acetyl procainamide ethobromide; APM, azidoprocainamide methoiodide; NPDA, *N*-pentyl deoxy-ajmalinium; NMN, *N*-methyl nicotinamide, SIM, standard incubation medium; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

Anyway, there is growing evidence that the uptake pathways for cardiac glycosides and bile salts are at least partly separate [1, 16, 17]. Interestingly, competitive interactions of both K-strophantoside and taurocholate with uptake of the organic cation *N*-pentyl deoxy-ajmalinium (NPDA) into hepatocytes have been suggested while these compounds also decreased photoaffinity labeling of hepatocyte plasma membranes using a photolabile azido-derivative of NPDA.*

Overlapping substrate specificity of organic cations for the uptake systems was demonstrated in some cases. For instance the uptake of the organic cation tri-*n*-butylmethylammonium (TBuMA) was inhibited by both cardiac glycosides and taurocholate but also by the type I organic cations PAEB and APM [18]. In fact TBuMA showed uptake characteristics of both type I and the type II compounds depending on the concentration range studied.

In the present study, it is shown that the kinetic parameters of TBuMA transport are compatible with a high affinity/low capacity transport system and a low affinity/high capacity transport system of which one is the type I uptake system. The second process responsible for TBuMA uptake is apparently a multispecific uptake system that may also accommodate taurocholate, cardiac glycosides and type II organic cations.

MATERIALS AND METHODS

Chemicals. [*methyl*-^{3}H]TBuMA (21 Ci/mmol) and TBuMA were synthesized in our laboratory, according to procedures described by Neef *et al.* [19]. Radiochemical purity was checked by TLC and exceeded 99%. The muscle relaxants [16*β*-*N*-*methyl*-^{14}C]ORG 6368 (57.3 mCi/mmol), [16*β*-*N*-*methyl*-^{14}C]vecuronium (58 mCi/mmol) and [16ß-*N*-*methyl*-^{3}H]ORG 9426 (9.9 Ci/mmol) (see Fig. 2) were synthesized by Dr F. Kaspersen from Organon Drug Metabolism Research Labs, Oss, The Netherlands. Radiochemical purity was checked by TLC in two solvent systems and exceeded 95% for vecuronium and 99% for ORG 9426 and ORG 6368. Vecuronium, ORG 6368 and ORG 9426 were kindly supplied by Organon, Oss, The Netherlands. [^{3}H]Taurocholate and [^{3}H]ouabain were provided by New England Nuclear (NEN, Boston, MA, U.S.A.). APM was synthesized previously as described in Ref. 20. Collagenase-H (type I) and *d*-tubocurarine were from the Sigma Chemical Co. (St Louis, MO, U.S.A.). K-Strophantoside was purchased from Roth (Karlsruhe, F.R.G.). PAEB was kindly donated by E. K. Squibb & Sons Inc. (Princeton, NJ, U.S.A.). Taurocholate was from Fluka Chemie AG (Buchs, Switzerland), Dextran T 40 was purchased from Pharmacia (Uppsala, Sweden). All other chemicals were from Merck (Darmstadt, F.R.G.).

* Müller M, Mol WEM, Kurz G and Meijer DKF, Hepatic uptake mechanisms for organic cations. Kinetic and photoaffinity labeling studies to characterize potential carrier proteins for amphipathic (type 2) cations. *Mol Pharmacol*, submitted.

Hepatocyte preparation

Hepatocytes were obtained by using a modification of the procedure of Ref. 21 as previously described by us [18]. Cells were finally collected in Standard Incubation Medium (SIM) containing (millimolar), NaCl, 118; KCl, 5; $MgSO_4$, 1.1; $CaCl_2$, 2.5; KH_2PO_4, 1.2; glucose, 10; HEPES, 10; $NaHCO_3$, 25; Dextran T 40, 1% (w/v), adjusted to pH 7.4. The viability of the cells was routinely tested. More than 85% of the cells excluded trypan blue (0.2%).

Uptake studies in hepatocytes. To determine the initial uptake velocity of the various radiolabeled compounds, 3 mL cells suspension in SIM (2.5–3.0 × 10^6 cells/mL) was preincubated for 30 min at 37° under continuous shaking and gassing with 95% O_2, 5% CO_2. The experiment started after addition of the radiolabeled compound and samples were removed from the cell suspension at 30 sec time intervals. The uptake of the compounds by the cells was rapidly stopped by dilution with 3 mL ice-cold Stop Solution, containing (millimolar), NaCl, 143; KCl, 5; $MgSO_4$, 1.1; $CaCl_2$, 2.5; KH_2PO_4, 1.2; HEPES, 10, adjusted to pH 7.4. The cells were separated from the medium by rapid filtration over Whatman GF/C filters under a constant vacuum of 600 mbar. The filters were washed twice with 3 mL of ice-cold Stop Solution. To determine uptake of the radiolabeled compound by the cells, radioactivity remaining on the filters was counted. The filters were transferred into scintillation vials and 3 mL Safe Fluor (Packard, Groningen, The Netherlands) was added. The vials were counted for 5 min in a Beckman LS 1800 Liquid scintillation counter. The uptake was calculated by correcting the measured values at 37° for the values at 0° at $t = 0$ (binding to the filter and the surface of the cells). The measurements using the filtration method were validated in our laboratories and were comparable with the silicon oil centrifugation method as described by us [18].

The effect of various compounds on the initial uptake of the radiolabeled compounds was determined by addition of the compounds 0.5 min prior to the start of the experiment. All compounds were dissolved in incubation medium.

Statistical analysis. Values are expressed as mean percentage uptake ± SEM. Each treatment was compared to the control in the same experiment using the paired Student's *t*-test. Differences were considered to be significant if $P < 0.05$.

RESULTS

To determine the involvement of potential uptake systems for the organic cation TBuMA in isolated rat hepatocytes, two concentrations of the compounds were used to study the subsequent characteristics for uptake. Calculated from the kinetic data from TBuMA [18] 1 μM of the compound resulted in a 95% contribution of the high affinity/low capacity system whereas 25 μM resulted in a 50/50 contribution of the high and low affinity uptake systems.

It can be seen from Fig. 1 that differences are observed at the two concentrations of TBuMA concerning the effects of K-strophantoside and

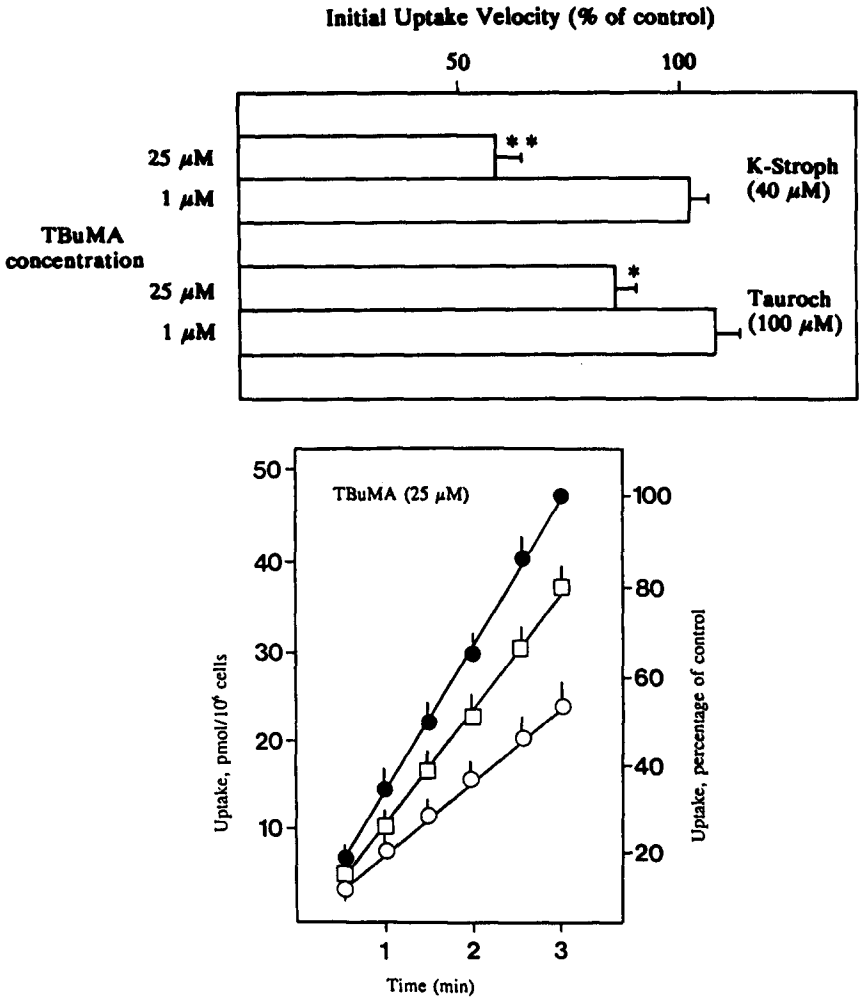

Fig. 1. Upper panel: effect of K-strophantoside (40 μM) and taurocholate (100 μM) on the initial uptake velocity of TBuMA (1 and 25 μM). Each bar represents the mean and SEM of at least three independent experiments, expressed as a percentage of the control in the same experiment. *$P < 0.05$ compared to control; **$P < 0.01$ compared to control. Lower panel: diagram, showing the uptake of TBuMA, at a concentration of 25 μM, expressed as pmol/10^6 cells (left axis) and as percentage compared to control (right axis). Control (●); in the presence of 40 μM K-strophantoside (○) or 100 μM taurocholate (□).

taurocholate (upper panel). The uptake of 1 μM TBuMA is not influenced in the presence of 40 μM K-strophantoside or 100 μM taurocholate, whereas the uptake of TBuMA at 25 μM is clearly affected by both compounds (see also Fig. 1, lower panel). A more pronounced effect on TBuMA uptake at 25 μM will be seen in the presence of 250 μM taurocholate [18]. The viability of the cells was not affected by the substrates and inhibitors used in this study, during the 3 min period of uptake.

To investigate whether the uptake system for TBuMA at 1 μM also accommodates endogenous cations, the effects of choline, thiamine and *N*-methyl nicotinamide (NMN) on TBuMA uptake at 1 μM were determined. From Table 1, it can be seen that only thiamine at a concentration 100 times the TBuMA concentration inhibits the initial uptake velocity of TBuMA significantly. This table further shows that the uptake of TBuMA at 1 μM is reduced in the presence of some monoquaternary and bisquaternary compounds and the Ca^{2+}-channel blocker verapamil. Especially at higher inhibitor/TBuMA ratios the reduction of the initial uptake velocity of TBuMA is pronounced.

To characterize the involvement of more than one carrier system for type II organic cations, the influence of several compounds on the uptake of the muscle relaxants ORG 9426, ORG 6368 and vecuronium were studied (for structural formulas see Fig. 2). From Table 2 it can be seen that the uptake of the type II compounds at both 1 and 25 μM were inhibited in the presence of 10 μM K-strophantoside. Interestingly, taurocholate did not influence the uptake of 1 μM of the muscle relaxant at all, whereas the uptake at 25 μM was significantly decreased. Involvement of the type I uptake system in transport of low concentrations of the muscle relaxants was examined by measuring the uptake in

Table 1. Uptake of [^{3}H]TBuMA at 1 μM in the presence of various endogenous and exogenous cations

	Concn (μM)	Mean ± SEM (%)
Endogenous cations		
Choline	4	98 ± 7
	40	104 ± 8
	100	100 ± 17
Thiamine	4	90 ± 17
	40	108 ± 9
	100	59 ± 13*
NMN	4	90 ± 6
	40	99 ± 12
	100	84 ± 11
Exogenous cations		
PAEB	4	55 ± 7†
	40	20 ± 4‡
	100	16 ± 1‡
APM	4	91 ± 12
	40	38 ± 4‡
	100	26 ± 3‡
Vecuronium	4	77 ± 11
	40	26 ± 8‡
	100	36 ± 4‡
d-Tubocurarine	4	70 ± 11*
	40	37 ± 3‡
	100	16 ± 2‡
Verapamil	4	41 ± 4‡
	40	12 ± 2‡
	100	6 ± 1‡

* $P < 0.05$.
† $P < 0.01$.
‡ $P < 0.001$.

the presence of high concentrations of the type I compound PAEB. It can be seen from Table 2 that PAEB did not inhibit the uptake of 1 μM of the muscle relaxants vecuronium, ORG 6368 and ORG 9426. The effects of the inhibitors at comparable conditions on the uptake of the type II compounds at 25 μM are also depicted in Table 2. It can be seen that at a concentration of 25 μM the uptake of the muscle relaxants are inhibited by both K-strophantoside and taurocholate.

To investigate the interrelation of the uptake systems for bile acids, cardiac glycosides and organic cations in isolated hepatocytes, mutual inhibition studies were performed. Therefore the effects of some organic cations on the initial uptake velocity of taurocholate and ouabain were observed. With respect to taurocholate, the compound is known to be taken up by the liver via a Na^+-dependent and a Na^+-independent pathway. The carrier system for Na^+-independent taurocholate transport was proposed to be multispecific. Interactions were described for a large variety of substances as cholate, bromosulfophthalein, steroids and cationic substances [16, 17, 22–25]. Therefore a concentration of taurocholate was chosen, close to the apparent K_m of the Na^+-independent uptake system, which is near 65 μM. The results of this study are depicted in Fig. 3, upper panel. It can be seen that the uptake of 65 μM taurocholate is clearly reduced in the presence of both the cardiac glycoside K-strophantoside (50 μM) and the bulky multivalent muscle relaxant ORG 9426 (200 μM). On the other hand, both PAEB and TBuMA (200 μM) only slightly but not significantly lowered the uptake of taurocholate. In the absence of Na^+ in the medium (Na^+ salts were replaced by Li^+ salts) TBuMA failed to further decrease the uptake of taurocholate (data not shown).

Finally, we performed mutual inhibition studies with respect to uptake of the cardiac glycoside ouabain. Figure 3, lower panel, shows that the initial uptake velocity of ouabain at 20 μM was decreased significantly in the presence of ORG 9426 (200 μM) and K-strophantoside (200 μM). Taurocholate exhibited an extremely strong inhibitory effect on the uptake of ouabain whereas TBuMA at 200 μM did not show a significant reduction in the uptake of ouabain. Only at 1 mM did TBuMA show a significant inhibitory effect. In addition, this concentration inhibited the uptake of ouabain at 150 μM, which is around its proposed K_m value [16], to the same extent (data not shown).

DISCUSSION

Interactions in the carrier-mediated hepatic uptake of cationic, anionic and uncharged compounds are not well understood. It was found that the uptake of some organic cations in hepatocytes, showed interactions with the uncharged cardiac glycosides and the anionic compound taurocholate as recently reviewed by us [1, 2]. Most of these interactions concerned the so called type II compounds. Uptake rate of type I compounds, such as PAEB and its derivatives were not significantly affected in the presence of the latter compounds.

Recently, we reported on the substrate specificity of the monovalent organic cation TBuMA. It was suggested that this compound is accommodated by both the type I and the type II cation uptake system [18].

In the present study, we investigated whether the kinetic parameters, found in this previous study, are compatible with the involvement of the two types of carrier systems. In other words, are the high affinity/low capacity system and the low affinity/high capacity system detected for TBuMA indeed identical to the type I and type II uptake system? To study this item, the uptake of TBuMA was investigated at a concentration near the K_m value of its high affinity system, which is 1 μM. On the basis of the kinetic parameters of TBuMA in isolated rat hepatocytes [18] it can be calculated that at this concentration more than 90% of the total uptake occurs via the high affinity/low capacity system. In the present study, the uptake characteristics of TBuMA at 1 μM were compared with those of 25 μM. At this concentration both the high affinity system and the low affinity system are equally involved in the uptake of TBuMA [18].

Because of the multiplicity of the overlapping transport systems involved (two or even more),

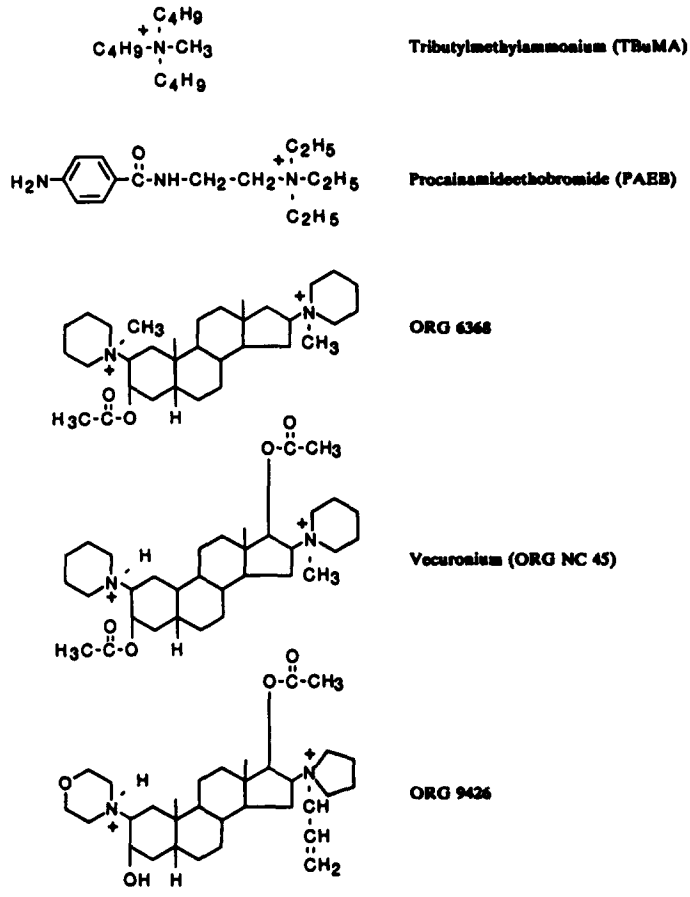

Fig. 2. Structural formulas of the cationic drugs used in this study. The tertiary amine groups in both vecuronium and ORG 9426 are protonated over 90% at physiological pH.

Table 2. Uptake of steroidal (bisquaternary) organic cations at a tracer dose and at 25 μM in the presence of various inhibitors

	Concn (μM)	Tracer concn	25 μM
		Mean ± SEM (%)	Mean ± SEM (%)
Vecuronium			
+ K-strophantoside	10	45 ± 12*	61 ± 13*
+ taurocholate	40	90 ± 14	73 ± 14
+ PAEB	40	126 ± 17	83 ± 12
ORG 9426			
+ K-strophantoside	10	25 ± 4‡	13 ± 1‡
+ taurocholate	40	116 ± 17	77 ± 9*
+ PAEB	40	111 ± 17	87 ± 27
ORG 6368			
+ K-strophantoside	10	7 ± 2‡	27 ± 4‡
+ taurocholate	40	84 ± 7	60 ± 5†
+ PAEB	40	93 ± 15	86 ± 12

* $P < 0.05$.
† $P < 0.01$.
‡ $P < 0.001$.

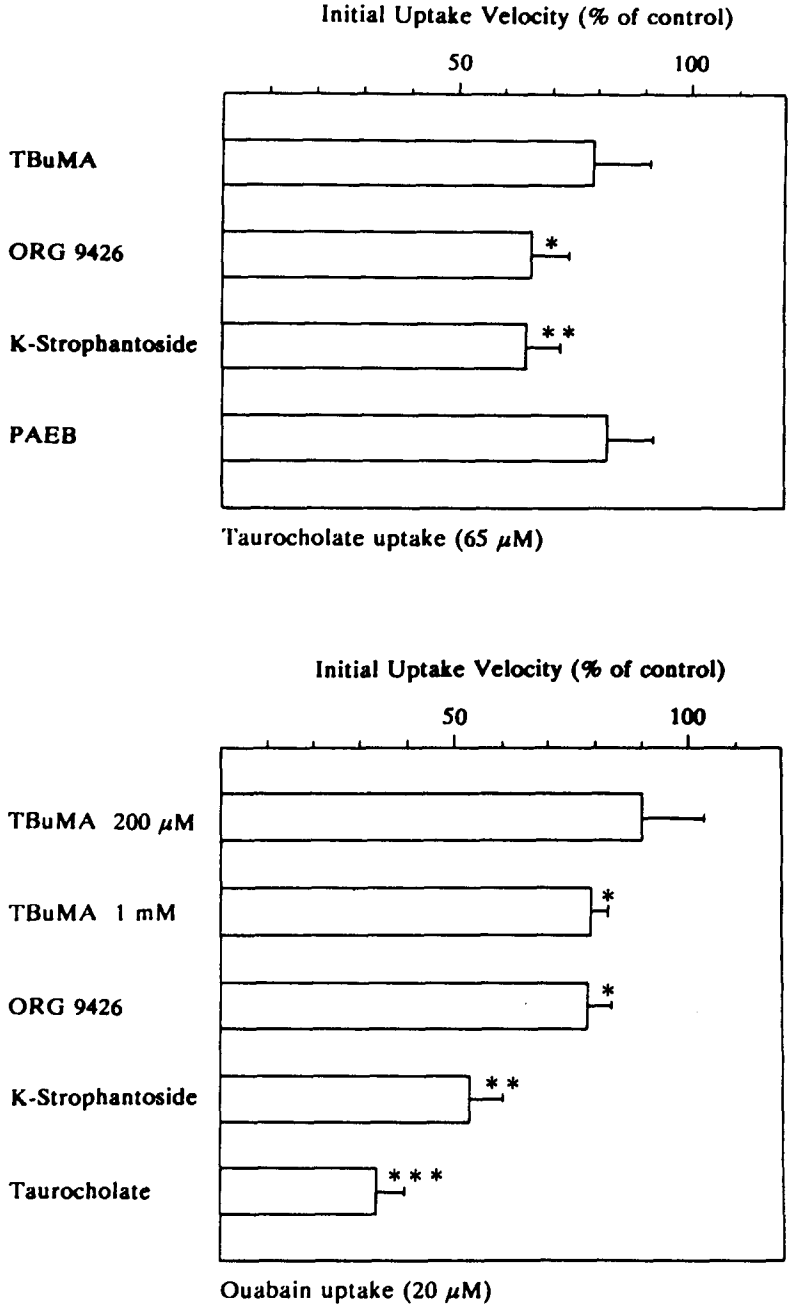

Fig. 3. Effects of various compounds on the initial uptake velocity of taurocholate (65 μM) (upper panel) and ouabain (20 μM) (lower panel). K-Strophantoside was present at a concentration of 50 μM, whereas the other inhibitors were present at concentrations of 200 μM, or 1 mM (TBuMA in lower panel). Each bar represents the mean and SEM of at least three independent experiments, expressed as a percentage of the control in the same experiment. *$P < 0.05$ compared to control; **$P < 0.01$ compared to control. ***$P < 0.001$ compared to control.

classical kinetic analysis yielding V_{max} and K_m parameters as well as establishment of competitive or non-competitive inhibition type are not feasible. The interpretation of such complicated graphs is often highly biased and does not allow a solid estimation of apparent K_m and V_{max} values even after sophisticated curve-fitting procedures and "weighing" of the individual data points. Unfortunately, there are no alternatives for such methods to date. Therefore, we simplified our method by measuring the uptake of the cation at only two different concentrations (at the relatively low and high concentration range).

From Fig. 1, upper panel, it can be seen that the

inhibition patterns at 1 μM TBuMA are completely different compared to those at 25 μM: K-strophantoside and taurocholate both reduced the uptake of TBuMA at 25 μM whereas the uptake at 1 μM TBuMA was not affected at all (see also Fig. 1, lower panel). This strongly indicates involvement of at least two uptake systems.

The question arose if the postulated carrier systems for endogenous cations such as thiamine, NMN and choline could be involved in the uptake of TBuMA. Table 1 shows the results on TBuMA uptake at 1 μm in the presence of the above-mentioned endogenous organic cations. Choline and NMN did not have a significant inhibiting effect on the uptake of TBuMA at 1 μM. Only thiamine reduced the uptake somewhat, although only at an inhibitor/substrate ratio of 100. In the reversed type of experiment TBuMA had little effect on choline (25 μM) uptake rate up to an inhibitor/substrate ratio of 4. This lack of interaction was confirmed by others showing that the uptake of both NMN and thiamine in isolated basolateral liver plasma membrane vesicles was not reduced by TBuMA at inhibitor/substrate ratios of 200 and 1000, respectively.* These data, combined with earlier findings that the uptake of TBuMA at 25 μM was not consistently affected in the presence of choline, NMN and thiamine [18] do indicate that TBuMA and the endogenous cations do not share a common carrier-mediated uptake system.

Pronounced interactions occurred with a wide variety of exogenous cationic agents. All the tested compounds showed a clear inhibiting effect on the uptake of TBuMA both at 25 and 1 μM. It is likely that at the low concentration range of TBuMA the type I cation system is responsible for its uptake. This is supported by the fact that other type I substrates like PAEB and its azido derivative APM clearly inhibited TBuMA uptake, whereas they failed to inhibit the uptake of type II compounds [3, 13]. In previous studies it was observed that the uptake of PAEB and APM are not basically affected by cardiac glycosides [6–8] or taurocholate [8]. Consequently PAEB, APM and TBuMA at the relatively low concentration, likely represent pure type I organic cations.

At first sight it seems surprising that type II organic cations of which transport is inhibitable by cardiac glycosides and bile salts (in contrast to type I compounds) can inhibit transport of the type I compounds. The question arose if such type II compounds at a relatively low concentration can also be transported by the supposed type I process. Therefore, we studied the uptake of low concentrations (tracer doses) of the muscle relaxants vecuronium, ORG 9426 and ORG 6368, all being hypothetized as type II compounds [1, 2, 13]. Table 2 shows that at a tracer concentration of the three muscle relaxants, K-strophantoside had a profound inhibitory effect in contrast to its effect on TBuMA uptake at 1 μM. In addition, high concentrations of PAEB did not affect the uptake of the muscle relaxants at this concentration range as was reported before vecuronium [13]. We conclude therefore that the type II organic cations also at low concentrations are not transported by the type I system. However vice versa, this does not exclude an influence of type II compounds on the type I uptake system: the type II compounds may strongly bind to the supposed type I cation carrier system without being transported by it.

The data from Table 2 exhibit another interesting phenomenon. The bile acid taurocholate did not inhibit the uptake of a tracer dose of the muscle relaxants, whereas it clearly affected uptake of these agents at 25 μM. Since the taurocholate concentration in both experiments was identical, at least two different carrier systems should be involved in the uptake of type II organic cations. The unequal inhibitory potency of taurocholate with regard to the type II compounds (Table 2) can well be explained by differences in affinity of the three compounds for the carrier systems involved in uptake [13].

The present data are indicative of the involvement of a carrier system responsible in the uptake of taurocholate, cardiac glycosides as well as the amphipathic cations. Multispecific uptake systems were earlier suggested for bile acids and bromosulfophthalein [16, 23, 24, 26], bile acids and ouabain [16, 17, 27], cholate and iodopamide and antamanide [28] and bile acids and steroids [29]. With regard to bile acids these studies mainly refer to the Na^+-independent pathway although recently multispecifity for the Na^+-dependent pathway was described [26]. The proposed involvement of the Na^+-independent taurocholate uptake system in the hepatic uptake of ouabain [17] is in line with the observation that uptake of the cardiac glycoside is basically Na^+-independent [16]. The K_m for taurocholate for the Na^+-independent carrier system is thought to be around 65 μM [22, 25]. To substantiate the existence of a multispecific uptake system we subsequently studied the influence of various agents on taurocholate and ouabain uptake rate. Figure 3 shows that the uptake of taurocholate at 65 μM is significantly inhibited in the presence of 200 μM of the muscle relaxant ORG 9426 and 50 μM of the cardiac glycoside K-strophantoside. In contrast PAEB did not reduce the uptake of taurocholate, which was anticipated since, vice versa, the uptake of PAEB and other type I compounds is not inhibited by taurocholate. TBuMA on the other hand, only slightly inhibited the uptake of taurocholate. Even in the absence of Na^+, the uptake of taurocholate could not be inhibited to a lower degree in the presence of TBuMA.

The cardiac glycoside uptake system was studied using radiolabeled ouabain. This compound is also capable of inhibiting the uptake of type II cations, although it is not as potent as K-strophantoside and digitoxin [11, 13]. Indeed K-strophantoside strongly reduced ouabain uptake (Fig. 3). Also the type II compound ORG 9426 decreased the uptake of ouabain significantly. The uptake of ouabain at 20 μM was also reduced considerably in the presence of taurocholate whereas TBuMA did not show a significant inhibition at an inhibitor/substrate concentration ratio of 4. Only at a ratio of 50, did TBuMA significantly reduce ouabain uptake. The uptake of ouabain near its supposed K_m value, i.e.

* Moseley RH, personal communication.

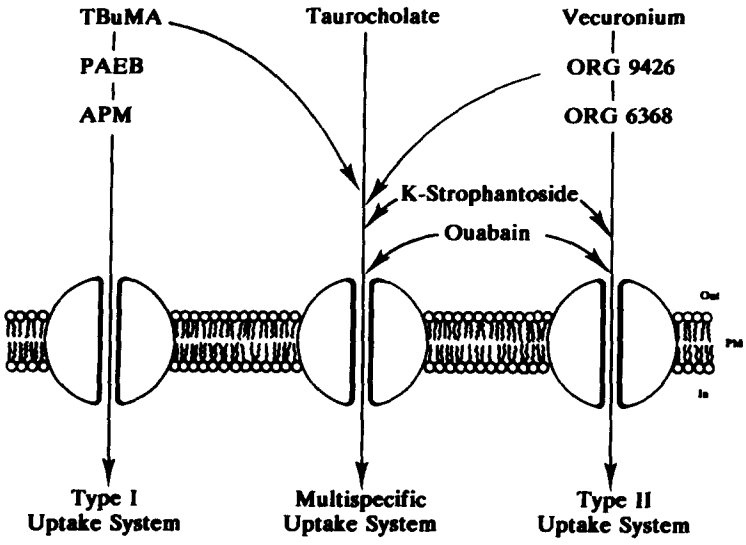

Fig. 4. Schematic diagram of carrier systems for exogenous organic cation uptake in the rat hepatocyte.

150 μM [16], was not further decreased in the presence of TBuMA at 1 mM (data not shown). It is tempting to suggest that the type II compounds, represented here by ORG 9426, share at least one common carrier system with bile salts and cardiac glycosides, since mutual interactions were observed. TBuMA may, apart from the type I and type II cation system, also be transported by this multispecific system although its weak inhibitory effects on ouabain and taurocholate uptake indicate a relatively low affinity for the particular carrier.

An earlier kinetic evaluation of a possible common carrier system for both ouabain and taurocholate led to the conclusion that these compounds do not share the same carrier system, since the uptake of taurocholate was not affected in the presence of ouabain [17]. In our study however, it was shown that K-strophantoside, a cardiac glycoside with a higher affinity for the uptake system, clearly inhibits the uptake of taurocholate at 65 μM.

In the light of the loss of polarity of the isolated rat hepatocytes (specific canalicular and sinusoidal plasma membrane domains will be lost after isolation), one wonders if the uptake systems investigated in the present study are all present in the sinusoidal region of the hepatocyte. In this respect it should be mentioned that most of the interactions described in the present study have also been observed in the isolated perfused rat livers. Taurocholate clearly interferes with the plasma disappearance of vecuronium [3] and of TBuMA [30]. K-Strophantoside strongly inhibits uptake of the type II compounds *d*-tubocurarine [6], hexafluronium [11], vecuronium [3] and TBuMA (data not shown).

From the present study it is concluded that organic cations can be taken up into the liver by a "multispecific" carrier system in addition to the type I and type II system (for a schematic diagram, see Fig. 4). Mutual transport interactions shown in the present study indicate that this carrier system also accommodates bile acids and cardiac glycosides. In contrast to PAEB and its derivatives, TBuMA clearly represents a mixed type of model compound. Depending on the concentration range applied it can in principle be transported by the type I cation system while at relatively high concentrations the above-mentioned multispecific carrier may also contribute to its overall hepatic uptake rate.

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