Biochemical Pharmacology, Vol. 38, No. 19, pp. 3223–3231, 1989.
Printed in Great Britain.

0006-2952/89 $3.00 + 0.00

HEPATOBILIARY TRANSPORT OF THE ANIONIC ORGANOMERCURY COMPOUND (MERSALYL) IS CARRIER MEDIATED

THERESE THALHAMMER and JÜRG GRAF
Department of General and Experimental Pathology, University of Vienna, Vienna, Austria

(*Received* 2 *September* 1988; *accepted* 31 *March* 1989)

Abstract—The hepatobiliary excretion of the anionic organic mercury compound (mersalyl) was studied in the isolated perfused rat liver and in isolated rat liver plasma membrane vesicles. In the isolated perfused liver, mersalyl is immediately taken up from the perfusion medium and concentratively excreted into bile. Uptake is characterized by saturation kinetics $(S)_{0.5} = 20\mu M$, $V_{max} = 117$ nmoles/min/g liver, cooperativity of mersalyl binding sites, stimulation by extracellular sodium and temperature dependence. Uptake of mersalyl into basolateral membrane vesicles also exhibits characteristics of a carrier mediated transport: saturation kinetics $(S)_{0.5} = 28\ \mu M$, $V_{max} = 1.6$ nmoles/min/mg protein, dependence on extravesicular sodium, cooperativity of mersalyl binding sites, temperature dependence and transstimulation by intravesicular non-radioactive mersalyl. Uptake was inhibited by α-naphthylacetic acid and mercapto group reagents, indicating involvment of mercapto groups on the carrier and a binding site for carboxylic anions. Data from the isolated perfused liver and from isolated basolateral vesicles indicate that mersalyl uptake into the liver is carrier mediated. Uptake mechanism and driving forces appear analogous to those for the uptake of chemically related compounds such as taurocholic acid. Therefore it is speculated that mersalyl may be transported by carrier molecules which apparently accept numerous chemically unrelated compounds.

The rate of biliary excretion of endogenous compounds and xenobiotics depends on several factors such as hepatic blood flow, bile flow rate, mechanisms of transport across the sinusoidal and the canalicular membrane barrier and on intracellular metabolism and conjugation (see Refs 1 and 2).

The liver is the main site of detoxification (biotransformation) of xenobiotics including organometallic compounds, the reaction products of which are predominantly secreted into bile [3]. Biliary secretion of mercury compounds has been studied [4–6] but so far the transport mechanisms involved have not been analysed.

Isolated liver plasma membrane vesicles derived from the basolateral and the canalicular membrane domain of the hepatocyte surface membrane have been employed to analyse hepatic uptake of amino acids [7, 8] and transcellular transport of taurocholic acid [9–13]. This technique allows the determination of transport kinetics and driving forces of individual membrane transport processes of substrates independently of other components involved in their biliary excretion.

This study investigates the mechanisms of hepatobiliary transport of mersalyl (*o*-(*N*-(3-hydroxymercury - 2 - methoxypropyl)carbamyl) - phenoxyacetic acid) an organic mercury anion which has previously been used as a diuretic. After intracellular conjugation, mainly with glutathione [14, 15], this compound is excreted into bile at extremely high concentrations.

We compare data obtained in the perfused liver with those obtained from transport studies in isolated membrane vesicles. It is shown that uptake of mersalyl across the basolateral membrane and excretion of mersalyl-glutathione across the canalicular membrane are both mediated by a membrane carrier. Competition experiments with other organic anions and mercaptogroup reagents indicate that the basolateral barrier exhibits a binding site for carboxylic ions as well as reactive mercapto groups. Basolateral uptake of mersalyl is stimulated by the presence of sodium and a transmembrane sodium gradient, whereas canalicular transport of the glutathione-conjugate is augmented by the transmembrane electrical potential.

MATERIALS AND METHODS

Isolated perfused liver. Surgery and liver perfusion experiments were carried out as described earlier [16] except that a non-recirculating (single pass) system was used. Perfusate flow to the isolated organ was from several reservoirs containing prewarmed (38°), gassed (95% O_2, 5% CO_2) solutions each being connected to the perfusate system via a valve. Control perfusion was adjusted to 3 ml/min/g liver. Experiments were started after 30 min control perfusion, a time required for the recovery of the organ from metabolic perturbations during surgery and for the stabilization of bile flow rate.

Hepatic uptake was calculated from the concentration differences between portal and hepatic venous perfusate, whereas biliary excretion of compounds was determined from biliary concentration

* Correspondence should be addressed to: Dr Therese Thalhammer, Department of General and Experimental Pathology, University of Vienna, Währingerstraße 13, A-1090 Vienna, Austria.

and bile flow, the latter being continuously monitored by the frequency and weight of single bile drops leaving the bile duct cannula. Each perfusion experiment was carried out at least in triplicate. For kinetic analysis average sinusoidal concentrations (c_{av}) were calculated according to Keiding *et al.* [17] by

$$c_{av} = (c_{in} - c_{out})/(\ln c_{in} - \ln c_{out})$$

where c_{in} is the concentration on the influent perfusate and c_{out} the concentration on the effluent perfusate.

Preparation of basolateral and canalicular plasma membrane vesicles. Membrane vesicles of basolateral origin were prepared as described by Van Amelsvoort *et al.* [18] by differential and sucrose density centrifugation from a rat liver homogenate. Membrane vesicles were collected at the 39.5/20% (w/v) interphase.

Canalicular membrane vesicles were isolated as originally described by Wisher and Evans [19] and the procedure was adapted in our laboratory [20]. After 10-min centrifugation of the diluted liver homogenate at 1000 *g*, the pellet was layered on a sucrose gradient and a crude membrane fraction was separated by rate zonal centrifugation in an A XII zonal rotor (MSE Manco Royal, Sussex, U.K.) at 3900 rpm for 45 min at the 38–45% (w/v) sucrose interphase. The fraction consisted mainly of basolateral and canalicular membranes and was washed in 1000 ml 1 mM $NaHCO_3$ at 7500 *g* for 20 min. Subfractionation of this crude membrane preparation was achieved after vigorous homogenization in a tight fitting type B Dounce homogenizer by further centrifugation in a discontinuous sucrose gradient, where canalicular membrane vesicles were obtained at an equilibrium density of 1.13. Vesicles were suspended in buffer (0.25 M sucrose, 0.2 mM $CaCl_2$, 10 mM Hepes/KOH, pH 7.5) to give a protein concentration of 5–10 mg/ml and stored on ice until use within the following 6 hr. Vesicles of both origin were assayed for marker enzymes as described in [20].

In basolateral membrane vesicles basolateral marker enzymes Na^+,K^+-ATPase and glucagon stimulated adenylate cyclase were enriched 10–15-fold, whereas canalicular markers alkaline phosphatase and Mg^{2+}ATPase were enriched only 3–6-fold above homogenate activities.

Canalicular vesicles were enriched 40–50-fold in Mg^{2+}ATPase, alkaline phosphatase, γ-glutamyltranspeptidase, 5'nucleotidase, whereas Na^+,K^+-ATPase and glucagon stimulated adenylate cyclase were 5–10-fold above homogenate activities.

The marker of the endoplasmatic reticulum glucose-6-phosphatase was 1.9- and 1.4-fold and the mitochondrial marker succinate-dehydrogenase was 0.9- and 0.8-fold above homogenate activities in basolateral and canalicular membrane vesicles, respectively.

Membrane orientation in vesicles was determined by freeze fracture analysis as reported in a previous paper [20]. We found that basolateral and canalicular membrane vesicles were at 60 and 80%, respectively in the inside–in configuration, which corresponds to the orientation of the membrane in the intact cell.

Transport assays. Uptake of radioactive labelled compounds into membrane vesicles was measured by a rapid filtration technique as described by Berner *et al.* [21].

In brief: 70 μl of isolated membrane vesicles were rapidly mixed with 70 μl incubation medium containing 0.25 M sucrose, 0.2 mM $CaCl_2$, 10 mM $MgCl_2$, 120 mM of an inorganic salt as described below, 10 mM Hepes/KOH, pH 7.5 and the radioactive labelled compound. Uptake was determined by rapid filtration of 20 μl aliquots of the reaction mixture through cellulose acetate filters (Sartorius SM 11306). The filters were washed once with 3 ml ice-cold washing buffer containing 0.25 M sucrose, 0.2 mM $CaCl_2$, 5 mM $MgCl_2$, 100 mM NaCl and 10 mM Hepes/KOH, pH 7.5. Radioactivity entrapped on the filter was measured by liquid scintillation counting.

To determine whether uptake of mersalyl reflects transmembrane movement or binding to the membrane surface the effect of medium osmolarity on vesicular mersalyl content was determined. The equilibrium content of mersalyl was measured in a transport medium containing different concentrations of sucrose.

Extrapolation to infinite high medium osmolarity shows that 80–90% of mersalyl is bound to membrane components. These values were obtained from a plot of 1/osmolarity (x) vs vesicular content (y) giving the following equations:

$y = 6.08 \pm 0.22 + 0.306 \pm 0.05x$ at 200 μM,
$y = 3.78 \pm 0.13 + 0.301 \pm 0.28x$ at 50 μM,
$y = 0.22 \pm 0.11 + 0.263 \pm 0.049x$ at 2.5 μM.

Initial binding was determined after 20-sec incubation of vesicles at 0° and amounted to 30% of initial uptake at 25°. There was no evidence that the fraction of 0° binding was reduced at low concentrations.

Data analysis and calculations. All experiments were repeated at least three times. If not stated otherwise, data in figures and tables were taken from individual experiments. Uptake measurements in isolated membrane vesicles were done in triplicate and values are expressed as means ±SD.

Synthesis of radiolabelled compounds. [^{14}C]Mersalyl–[^{35}S]glutathione was prepared by conjugation of [^{14}C]mersalyl with [^{35}S]glutathione by mixing equimolar amounts (1 mmol) in 10 mM Hepes/KOH at pH 7.5 under N_2 atmosphere (compare with Ref. 9). Completeness of reaction and absence of parent compounds was analysed by autoradiography on TLC plates (No. 5577, Merck, Darmstadt, F.R.G.) with *n*-propanol/H_2O (70:30, v/v) and *n*-propanol/NH_3 (70:30, v/v) as solvent.

α-Naphthyl[^{14}C]acetic acid was prepared from 1-(chloromethyl)-naphthaline and $^{14}CO_2$ by Grignard reaction. $^{14}CO_2$ was prepared *ad hoc* from Ba[^{14}C]CO_3 by addition of HCl.

Synthesized products were identified by NMR and i.r. spectroscopy and by TLC radio-chromatography revealing a purity >98%.

Materials. [^{14}C]Mersalyl (*o*-(*N*-(3-hydroxymercury- 2- methoxy- propyl- [^{14}C]carbamyl)- phenoxy)-acetic acid) was obtained from the Commissariat a L'Energie Atomique (CEA, Gif-Sur-Yvette,

Table 1. Uptake, biliary excretion and stimulation of bile flow by different mersalyl concentrations

c_{in} (μM)	c_{av} (μM)	Uptake (nmoles/min/g)	Biliary excretion* (nmoles/min/g)	Stimulation of bile flow (% of control)
5	3.4 ± 0.2	9.4 ± 0.4	5.2 ± 0.7	105.1 ± 3.9
10	6.3 ± 0.6	20.7 ± 2.1	11.2 ± 0.9	121.6 ± 6.0
25	15.7 ± 1.3	52.2 ± 4.2	27.1 ± 2.9	129.3 ± 5.5
50	34.8 ± 3.6	88.5 ± 9.2	44.2 ± 3.4	141.6 ± 8.4
60†	40.9 ± 4.4	91.9 ± 9.9	53.9 ± 6.1	148.4 ± 9.6
100†	75.6 ± 6.1	106.1 ± 8.5	75.3 ± 12.4	136.5 ± 12.1

Uptake and bile flow stabilized 3 min after shifting to the mersalyl-containing medium. In the experiments marked † uptake and bile flow gradually decreased after 5 to 10 min perfusion with the indicated concentrations and maximal rates given.

* Calculated from the radioactivity in bile/specific activity of the compound in the perfusion medium.

Means of 3 expts ± SD.

France) and [^{35}S]glutathione from the New England Nuclear Corp. (Boston, MA). Ba[^{14}C]CO_3 was a gift from the Kernforschungszentrum Seibersdorf (Austria). Mersalyl, *p*-chloromercuribenzoic acid, $HgCl_2$, *N*-ethylmaleimide, 2,3-dimercaptopropanol, 1-(chlormethyl)-naphthaline were obtained from Sigma (Deisenhofen, F.R.G.) and α-naphthylacetic acid from Donau Chemie (Linz, Austria). All other chemicals were purchased from E. Merck (Darmstadt, F.R.G.) and were of analytical grade.

RESULTS

Uptake of [^{14}C]*mersalyl and biliary excretion of radioactive metabolites in the isolated perfused liver*

Table 1 shows that steady-state uptake, biliary excretion and bile flow increase with increasing concentrations of mersalyl (5–100 μM) in the influent perfusate. Apparent toxic effects on isolated liver, resulting in an inhibition of bile flow and a decrease in uptake rates are noted during prolonged perfusion with concentrations above 50 μM. As revealed by TLC bile contains the glutathione conjugate (30%), free mersalyl (45%) and unidentified compounds, the latter possibly reflecting degradation products of the secreted conjugate (compare Refs 14 and 15). For the biliary secretion mechanism the influence of ductular modifications cannot be excluded. Therefore excretion was studied in isolated canalicular vesicles.

At the concentrations employed uptake of mersalyl into liver shows saturability as previously found for other organic mercury compounds [22]. It is noted though that the uptake below 10 μM is lower than expected for a first-order Michaelis–Menten kinetic [23]. Kinetic analysis of the data by linearization (Eadie, Hofstee and Hill plots; see Ref. 23) reveals a Hill coefficient of 1.5 (Fig. 1A) and half saturation $(S)_{0.5}$ at 20 μM with a maximal uptake rate (V_{max}) of 117 nmoles/min/g liver. This observation indicates that membrane transport is accomplished by a membrane carrier, being as effective as the carrier-mediated uptake of taurocholic acid [24]. The data also indicate cooperativity of binding sites for mersalyl which may either result in facilitated carrier–substrate interaction or in stimulation of transport velocity. In order to determine the nature of binding sites for mersalyl, inhibition of uptake by analogous compounds was studied. These included α-naphthylacetic acid (with respect to arylacetic acid structure) and other mercury compounds. As shown in Table 2 uptake of [^{14}C]mersalyl is inhibited by α-naphthylacetic acid and also, uptake of α-naphthyl-[^{14}C]acetic acid is mutually inhibited by the presence of mersalyl in the perfusion medium.* Other organic anions without the arylacetic acid structure, such as phenolphthalein (20 μM) and sulfobromophthalein (20 μM), had no influence on mersalyl uptake, but sulfobromophthalein (BSP) at a concentration of 20 μM inhibited biliary secretion of 2 μM mersalyl (biliary excretion: control 1.69 ± 0.28 nmoles/g/min, BSP addition 0.54 ± 0.18 nmoles/g/min). Experiments with mercapto groups reagents in the perfused liver apparently add to the toxicity of mersalyl, resulting in an inhibition of bile flow rate. Inhibition experiments with these compounds were therefore carried out in isolated plasma membrane vesicles only.

Uptake of mersalyl into the perfused organ is inhibited by low perfusate sodium concentration. As shown in Fig. 2 stepwise replacement of sodium by choline results in a gradual inhibition of uptake. Fifteen per cent of uptake appeared independent on extracellular sodium. The sodium-dependent component of mersalyl uptake shows saturability with respect to perfusate sodium concentration exhibiting a $(S)_{0.5}$ at a sodium concentration of 13.9 mM. Extracellular sodium may either affect the carrier substrate affinity or the transport velocity, or the transmembrane sodium concentration gradient may provide a

* In the isolated perfused rat liver uptake of α-naphthyl-[^{14}C]acetic acid is characterized by saturation kinetics ($(S)_{0.5}$ = 21 μM, V_{max} = 120 nmoles/min/g liver. Uptake is further dependent on extracellular sodium (75 and 80% inhibition in the absence of sodium, sodium being replaced by K^+ and choline, respectively). Uptake is augmented at an acidic extracellular pH (40% increase at pH 6.8) possibly indicating non-ionic diffusion.

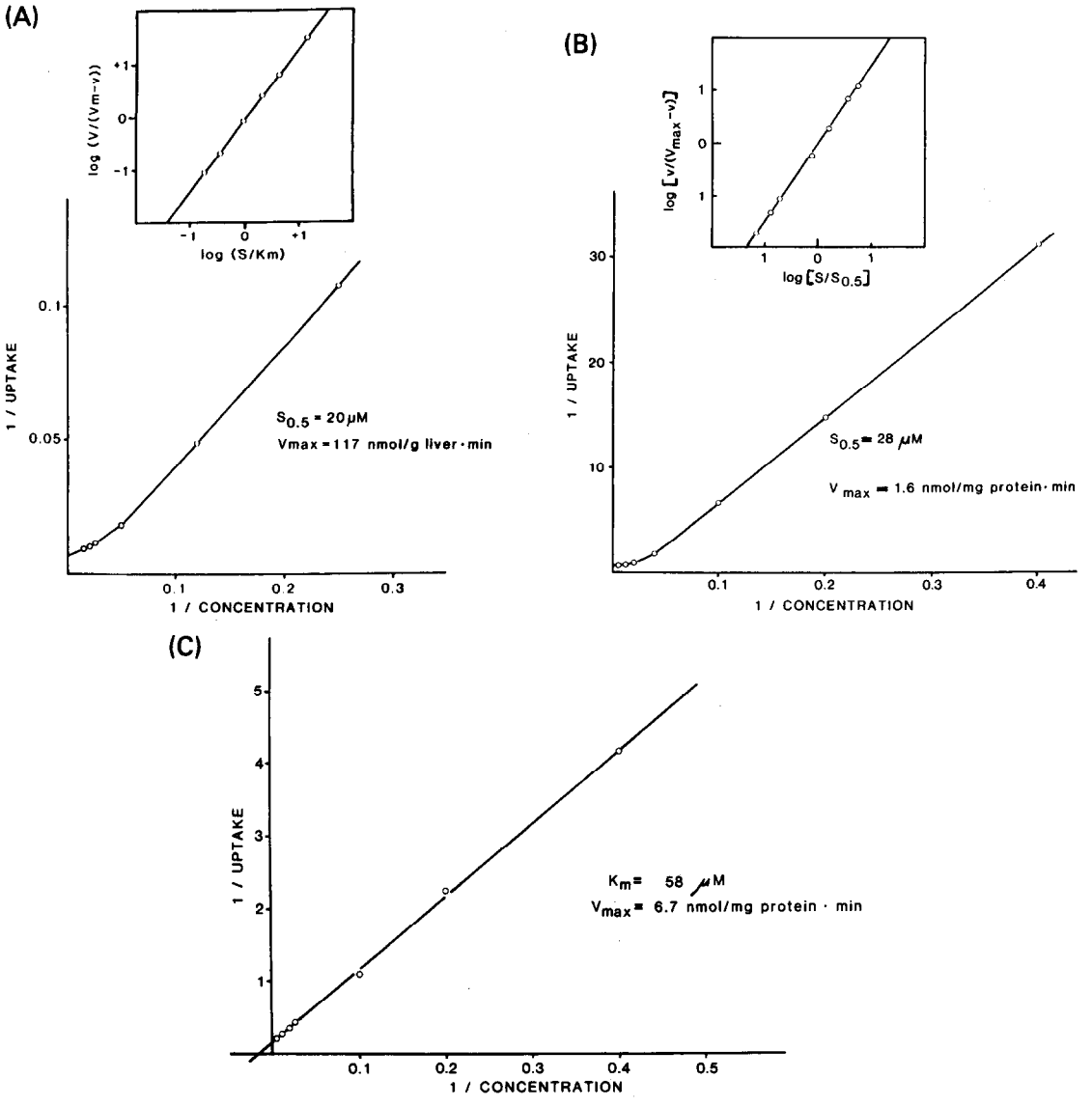

Fig. 1. Saturation kinetics of [^{14}C]mersalyl uptake in the isolated perfused liver (A) and in isolated basolateral membrane vesicles (B) and of mersalyl–glutathione in isolated canalicular membrane vesicles (C). Uptake in the liver was calculated after 3-min perfusion with [^{14}C]mersalyl. Uptake in isolated basolateral and canalicular plasma membrane vesicles was calculated from the vesicular content after 20-sec incubation at 25° minus the vesicular content at 0° determined in parallel experiments.

driving force for a sodium–substrate cotransport. The latter possibility was studied in isolated membrane vesicles.

Uptake of [^{14}C]*mersalyl in isolated basolateral plasma membrane vesicles*

Membrane binding, in addition to membrane transport, has been observed for various substrates (taurocholic acid [9–13], *l*-alanine [7, 18, 25] or glutathione (8, 26). This phenomenon was also encountered for mersalyl and was analysed in two sets of experiments.

Uptake of mersalyl into basolateral membrane vesicles (representing mainly unidirectional influx measurements) exhibits a different time course at 25° and at 0°. At 0° a rapid initial uptake is complete within 20 sec and further incubation (up to 120 min) does not result in additional accumulation of the compound. At 25° a rapid initial uptake is followed by progressive accumulation into vesicles, equilibrium values being obtained after 30 min. Rapid initial uptake at 0° and part of uptake at 25° appear to be due to extravesicular binding. Binding of the compound to vesicular membranes is further substantiated by determining the osmotically active intravesicular space and a comparison to 20-sec uptake values indicates that at equilibrium 80–90% of mersalyl is bound to membrane components either from the extra- or the intravesicular side. Initial binding (vesicular content after 20-sec incubation at

Table 2. Influence of α-naphthylacetic acid, phenolphthalein and sulfobromophthalein on uptake of [^{14}C]mersalyl and of mersalyl on uptake of [^{14}C]α-naphthylacetic acid in the isolated perfused liver

Addition to perfusion medium	[^{14}C]Mersalyl uptake nmoles/min/g liver
None	3.68 ± 0.23*
20 μM α-naphthylacetic acid	1.88 ± 0.41*
20 μM phenolphthalein	3.65 ± 0.43
20 μM sulfobromophthalein	3.45 ± 0.62
	[^{14}C]α-Napthylacetic acid uptake nmoles/min/g liver
None	2.18 ± 0.68*
20 μM mersalyl	1.02 ± 0.40*

Uptake values were obtained after 3 min perfusion with either 2 μM [^{14}C]mersalyl or 2 μM [^{14}C]α-naphthylacetic acid in presence of indicated compounds.

Means of 4 expts ± SD, *$P < 0.05$.

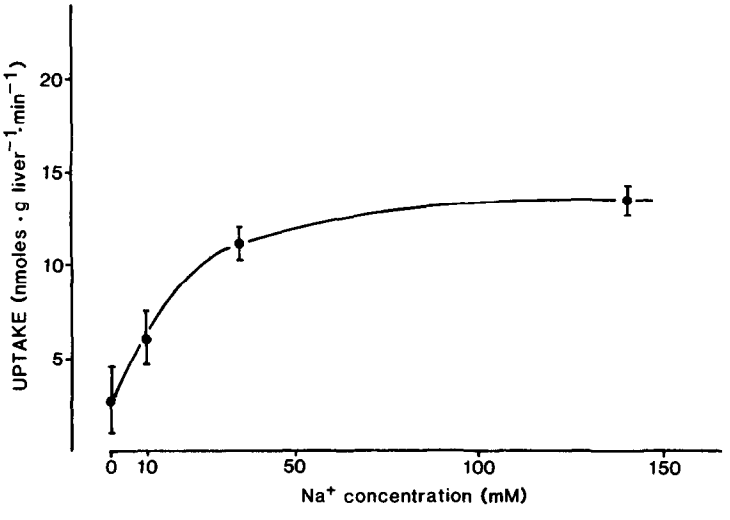

Fig. 2. Stimulation of mersalyl uptake in the isolated perfused liver at different Na^+ concentrations. Uptake values were obtained after 3-min perfusion with 10 μM [^{14}C]mersalyl. Na^+ was replaced in equimolar amounts by choline.

0°) was therefore determined in each experiment. This value was subtracted from uptake values at 25° when transmembrane transport function was assayed.

Transport of mersalyl into basolateral membrane vesicles exhibits saturation kinetics within the concentration range studied (2.5–250 μM). As already seen in the perfused liver, uptake into vesicles at low concentrations (below 10 μM) is lower than expected for first-order kinetics, revealing also a Hill coefficient of 1.5 (Fig. 1B). From the corresponding Eadie–Hofstee plot $(S)_{0.5}$ = 28 μM and V_{max} of 1.6 nmoles/min/mg protein were calculated, values in good agreement with those obtained for uptake in the perfused organ.

Extravesicular sodium concentration stimulates uptake of mersalyl into basolateral membrane vesicles. This was analysed at a mersalyl concentration of 200 μM in the presence of 120 mM NaSCN or 120 mM KSCN either outside the membrane alone or 60 mM on both sides of the membrane. Compared to KSCN, NaSCN on both sides stimulates temperature-sensitive initial uptake by 30%, whereas an increase by 40% is seen when NaSCN was only present in the extravesicular medium (these two values being statistically different at a level of $0.2 > P > 0.1$; Student's *t*-test). These data suggest, that extravesicular sodium increases substrate affinity and, in addition, that the transmembrane sodium gradient provides a driving force for substrate transport.

Uptake of mersalyl is subjected to transstimulation, i.e. preloading of membrane vesicles with non-radioactive mersalyl increases uptake of the radiolabelled compound. Under this condition uptake is also stimulated by the presence of sodium (Fig. 3).

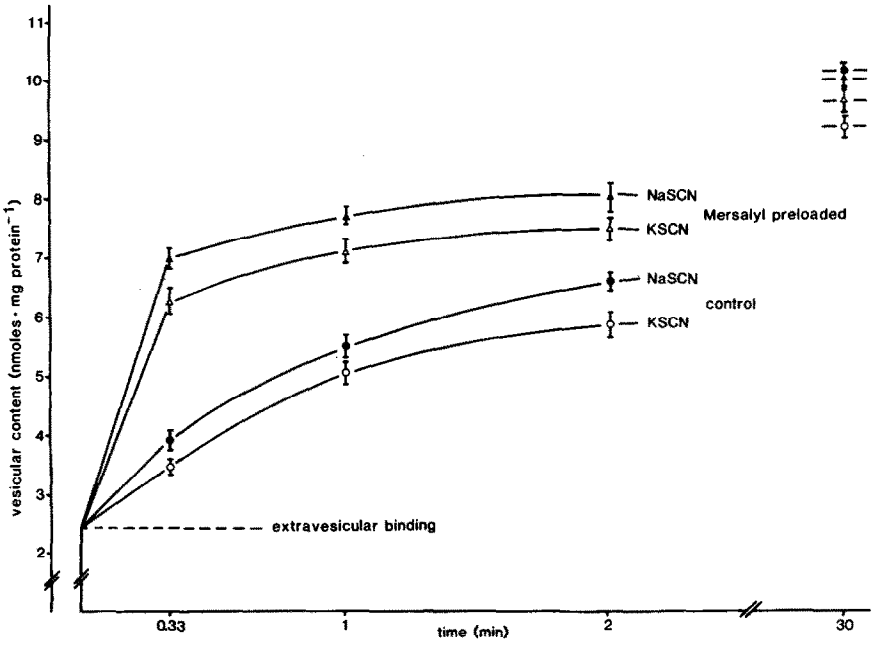

Fig. 3. Transstimulation of [^{14}C]mersalyl uptake into basolateral plasma membrane vesicles. Uptake of 200 μM [^{14}C]mersalyl was measured. Vesicles were preincubated 30 min at 25° either with or without (control) 200 μM non-radioactive mersalyl. 60 mM NaSCN or 60 mM KSCN were present in the extra- and intravesicular medium. Extravesicular binding was calculated from the initial uptake at 0° (2.4 ± 1.1 nmoles/mg protein)

Table 3. Effects of α-naphthylacetic acid and mercapto-group reagents on [^{14}C]mersalyl uptake into basolateral plasma membrane vesicles

Addition to incubation medium	Uptake (% of control)
200 μM α-naphthylacetic acid	56.1 ± 3.9
200 μM *p*-chloromercuribenzoic acid	48.3 ± 5.4
100 μM $HgCl_2$	46.7 ± 6.2
100 μM *N*-ethylmaleimide	49.2 ± 7.6

Uptake of 200 μM [^{14}C]mersalyl (100%) was determined after 20 sec incubation at 25° in the presence of indicated compounds. Incubation medium contained 120 mM NaSCN.

Means of triplicate experiments ± SD.

Significant transstimulation ($P > 0.05$) is also seen, when α-naphthylacetic acid is incorporated into vesicles (uptake was studied with 200 μM mersalyl outside and 200 μM α-naphthylacetic acid inside the vesicles, data not shown).

As shown in Table 3 uptake of mersalyl is inhibited by compounds which may either bind or compete for the carrier (*p*-chloromercuribenzoic acid, $HgCl_2$, *N*-ethylmaleimide or α-naphthylacetic acid). 2,3-Dimercaptopropanol (100 μM) which reacts with the mercury atom of mersalyl completely inhibits uptake. This data substantiate the observations made in the perfused organ that uptake of mersalyl is accomplished by a membrane carrier, interacting with the compound by two binding sites.

It was also attempted to determine an electrogenic component of mersalyl uptake by varying the electrical potential by applying transmembrane concentration gradients of permeant and inpermeant anions. Results indicate, that an intravesicular negative electrical potential stimulates uptake, but NMR data also show, that the labile R–Hg–OH group reacts with various anions in the incubation medium, so these experiments were discontinued.

Transport of [^{14}C]*mersalyl*-[^{35}S]*glutathione in isolated canalicular membrane vesicles*

Uptake of [^{14}C]mersalyl–[^{35}S]glutathione into canalicular membrane vesicles was studied at 0° and 25°. Initial uptake is rapid at 0°, but in contrast to basolateral membrane vesicles, it is not complete within 20 sec. Uptake continues during the following 30 min indicating that, in addition to extravesicular binding, slow temperature insensitive accumulation in vesicles occurs. At 25° rapid uptake of mersalyl–glutathione is observed, 70% of equilibrium values being already obtained within the first 20 sec. This temperature dependence indicates uptake by a membrane carrier, whereas uptake at 0° is in addition to initial binding, indistinguishable from accumulation by diffusion. As shown in Fig. 1C uptake at 25° exhibited saturation kinetics. Within a concentration range of 1–250 μM mersalyl–glutathione a linear Lineweaver–Burk plot is obtained revealing a $(S)_{0.5}$ of 58 μM; and a V_{max} of 6.7 nmoles/min/mg protein, which no indication of more than one binding site (see Fig. 1C).

Carrier-mediated transport across the canalicular

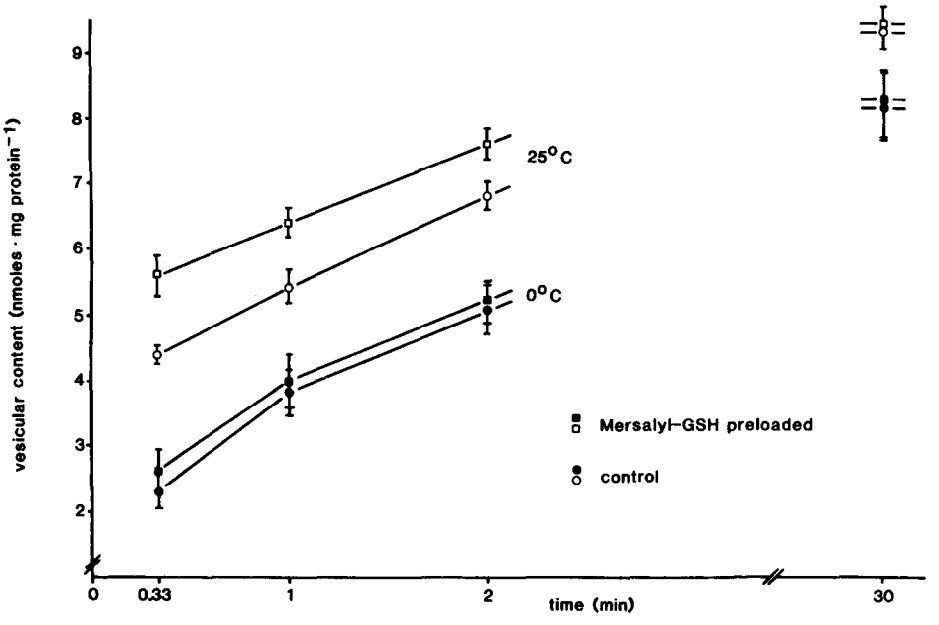

Fig. 4. Dependence of [^{14}C]mersalyl-[^{35}S]glutathione uptake on temperature and transstimulation with the non-radioactive compound. Extravesicular medium contained 200 μM [^{14}C]mersalyl-[^{35}S]glutathione. Before uptake measurements, vesicles were preincubated 30 min at 25° either in presence of 200 μM non-radioactive mersalyl-glutathione or in absence (control). Uptake was measured either at 25° or at 0°.

membrane is also demonstrated by transstimulation as shown in Fig. 4, where preloading of canalicular membrane vesicles with non-radioactive mersalyl-glutathione increases uptake of the labelled compound.

Dependence of transport on the transmembrane electrical potential is indicated by comparing uptake under two experimental conditions, one rendering the intravesicular potential positive with respect to the outside medium by an inward directed potassium gradient (120 mM K-gluconate outside in presence of 1 mg/ml valinomycin at K^+-ionophore) the other providing an inside negative potential by an inward directed SCN^- gradient (120 mM KSCN outside, absence of valinomycin). At 200 μM extravesicular concentration of mersalyl-glutathione, 1 min uptake values were 7.24 ± 0.34 nmoles/mg protein and 6.48 ± 0.33 nmoles/mg protein, respectively ($P < 0.05$), indicating that an intravesicular positive potential stimulates uptake. No significant difference between uptake values were observed when extravesicular KSCN (120 mM) was replaced by NaSCN (120 mM) or NaCl (120 mM).

DISCUSSION

Mersalyl is an organometallic agent reacting with mercapto groups. It is well known as an inhibitor of various enzymes and transport processes, including Na^+,K^+-ATPase [27] and the sodium-dependent *l*-alanine transport in isolated basolateral rat liver plasma membrane vesicles [24]. This compound has previously been used as a diuretic, but it has also a choleretic effect comparable to other organic anions, which are secreted into bile.

It was the aim of the present study of analyse hepatic transport of this toxic compound and the biliary excretion of its glutathione-conjugate.

In the isolated perfused liver and in basolateral membrane vesicles uptake of mersalyl exhibits similar characteristics: (1) saturation kinetics with comparable values of $(S)_{0.5}$; (2) cooperativity of mersalyl binding sites; (3) stimulation by extra(cellular)-vesicular sodium; (4) inhibition by the presence of another arylacetic acid (NAA).

Studies in isolated membrane vesicles further indicate, that the carrier is equipped with a binding site for both, the acetic group and the mercury atom of the compound. These binding sites appear also available from inside the cell membrane resulting in transstimulation of uptake of [^{14}C]mersalyl, when either the non-radioactive compound or α-naphthylacetic acid are incorporated into vesicles.

Our experiments in the perfused liver would be consistent with an inhibition of the Na^+-dependent fraction of mersalyl uptake by NAA, but studies in vesicles indicate that inhibition concerns both the Na^+-dependent and Na^+-independent fraction (see Table 3). However these findings were not studied in more detail. Vesicle studies also indicate that a transmembrane sodium gradient may serve as a driving force for uptake of the compounds into liver cells, but the weak effect of the sodium gradient also indicates that in the liver other mechanisms may sustain uptake, e.g. rapid intracellular binding or conjugation may maintain a transmembrane gradient of the compound. These arguments also apply for the partial sodium dependence of uptake of endogenous compounds such as bile acids and amino acids.

The major point of the experiments done with

canalicular membrane vesicles was to demonstrate that transport is carrier mediated. This was obviously difficult to do since the osmotically available space accounts only for approximately 5% of total measured uptake of mersalyl–GSH. The vast majority of uptake thus represents intravesicular binding. None the less, saturation kinetics, temperature dependence and transstimulation could be clearly demonstrated and show that uptake proceeds by a carrier-mediated process. Transstimulation indicates that transport properties across the membrane may be symmetrical. This appears worth noting since uptake into right side out canalicular vesicles is in the reverse direction of physiological flow. The stimulatory effect of a transmembrane electrical potential gradient was weak and exhibited no overshoot with respect to the high intravesicular binding. In addition an apparent diffusional transport component was found, but not further investigated.

For the investigations of hepatobiliary excretion of mersalyl and its glutathione-conjugate in isolated plasma membrane vesicles there were some technical limitations. First the non-ideal purity of our membrane vesicle preparation and the high degree of binding of the compounds studied. Principal limitations include the possibility that membrane transport processes may be altered during the vesicle isolation procedure and, with respect to the transcellular transport in the intact cell, the lack of knowledge of the magnitude of the driving forces and of other factors such as intracellular compartmentalization, and intracellular and bile micelle binding, all of which may sustain or alter the transcellular transport rate. In spite of this the data show that mersalyl and its glutathione conjugate are transported across the basolateral and canalicular cell membrane, respectively, by high-affinity carrier-mediated transport processes. In this regard transcellular movement of the compound exhibits similarities to the mechanisms of taurocholic acid and glutathione secretion [13].

Putative carrier proteins for hepatic uptake of various compounds have been recently identified and it is possible that uptake of mersalyl is accomplished by the non-selective transport protein [28]. In this case the presence on the carrier of a binding site for the arylacetic acid and a mercapto group binding site would have to be postulated.

On the other hand, at the canalicular membrane, carrier mediated transport of glutathione conjugates has been identified [29] and this carrier protein may be responsible for the secretory transport for mersalyl-GSH.

Acknowledgements—The work was supported by the Austrian Science Research Fonds (Project 6102). The authors thank Mr P. Wyskowsky for excellent technical assistance.

REFERENCES

1. Levine WG, Biliary excretion of drugs and other xenobiotics. *Ann Rev Pharmacol Toxicol* **18**: 81–96, 1978.
2. Levine WG, Biliary excretion of drugs and other xenobiotics. *Prog Drug Res* **25**: 361–420, 1981.
3. Smith RL, *The Excretory Function of Bile*. Wiley, New York, 1973.
4. Refsvik T and Norseth T, Methyl mercury compounds in rat bile. *Acta Pharmacol Toxicol* **36**: 67–78, 1975.
5. Tichy M, Horejsi M and Cikrt M, Effect of some chelating agents on the biliary excretion of mercury. 2. Relationship between the excretion of mercury and its binding to bile fractions *J Hyg Epidem Microbiol Immunol* **24**: 309–323, 1980.
6. Ballatori N and Clarkson TW, Biliary transport of glutathione and methylmercury. *Am J Physiol* **244**: G435–G441, 1983.
7. Van Amelsvoort JMM, Sips HJ, Apitule MEA and van Dam K, Heterogenous distribution of the sodium dependent alanine transport activity in the rat hepatocyte plasma membrane. *Biochem Biophys Acta* **600**: 950–960, 1980.
8. Inoue M, Kinne R, Tran T and Arias IM, Glutathione transport across hepatocyte plasma membranes. Analysis using isolated rat liver sinusoidal membrane vesicles. *Eur J Biochem* **138**: 491–495, 1984.
9. Inoue M, Kinne R, Tran T and Arias IM, Taurocholate transport by rat liver sinusoidal membrane vesicles: evidence of Na^+ cotransport. *Hepatology* **2**: 572–579, 1982.
10. Ruifrok PG and Meijer DKF, Sodium coupled uptake of taurocholate by rat liver membrane vesicles. *Liver* **2**: 28–34, 1982.
11. Duffy M, Blitzer BL and Boyer JL, Direct elimination of the driving forces for taurocholate uptake into rat liver plasma membrane vesicles. *J Clin Invest* **72**: 1470–1481, 1983.
12. Blitzer BL and Donovan CB, A new method for the rapid isolation of basolateral plasma membrane vesicles from rat liver, characterization, validation and bile transport studies. *J Biol Chem* **259**: 9255–9301, 1984.
13. Meier PJ, Meier-Abt ASt, Barret C and Boyer JL, Mechanism of taurocholate transport in canalicular and basolateral rat liver plasma membrane vesicles. *J Biol Chem* **259**: 10614–10622, 1984.
14. Weiner IM and Müller OH, A polarographic study of mersalyl (salyrgan)-thiol complexes and of the excreted products of mersalyl. *J Pharmacol Exp Ther* **113**: 241–249, 1955.
15. Leuschner F, Ausscheidungsprodukte des Salyrgans im Harn. *Arch Exp Pathol Pharmacol* **230**: 513–523, 1957.
16. Graf J and Peterlik M, Oubain mediated sodium uptake and bile formation by the isolated perfused rat liver. *Am J Physiol* **23**: G876–885, 1976.
17. Keiding S, Johansson S, Winkler K, Tonnesen K and Tygstrup N, Michaelis–Menten-Kinetic of galactose elimination by the isolated perfused pig liver. *Am J Physiol* **230**: G1302–1313, 1976.
18. Van Amelsvoort JMM, Sips HJ and van Dam K, Sodium-dependent *l*-alanine transport in plasma membrane vesicles from rat liver. *Biochim Biophys Acta* **600**: 950–960, 1978.
19. Wisher MH and Evans WH, Function polarity of the rat hepatocyte surface membrane. Isolation and characterization of plasma membrane subfractions from the blood sinusoidal, bile canalicular and contiguous surface of the hepatocyte. *Biochem J* **146**: 375–388, 1975.
20. Thalhammer T, Hansel G and Graf J, Analysis of hepatic uptake and biliary excretion of an anionic xenobiotic utilizing isolated plasma membrane vesicles. In: *Pharmacochemistry Library*, Vol. 8 (Ed. Tichy M), pp. 419–424. Elsevier, Amsterdam, 1985.
21. Berner W, Kinne R and Murer H, Phosphate transport into brush border membrane vesicles from rat small intestine. *Biochem J* **160**: 467–474, 1976.
22. Fang SC and Fallin E, Uptake and subcellular cleavage of organomercury compounds by rat liver and kidney. *Chem Biol Interact* **9**: 57–64, 1974.

23. Atkinson DE, *Cellular Energy Metabolism and its Regulation.* Academic Press, New York, 1977.
24. Reichen J and Paumgartner G, Uptake of bile acids by the perfused rat liver. *Am J Physiol* **231**: 734–742, 1976.
25. Sips HJ, van Amelsvoort JMM and van Dam K, Amino acid transport in plasma membrane vesicles. Characterization of *l*-alanine transport. *Eur J Biochem* **105**: 217–223, 1982.
26. Inoue M, Kinne R, Tran T and Arias IM, The mechanism of biliary secretion of reduced glutathione. Analysis of transport process in isolated rat liver canalicular membrane vesicles. *Eur J Biochem* **134**: 467–471, 1983.
27. Taylor CB, The effect of mercurial diuretics on adenosine triphosphatase of rabbit kidney *in vitro*. *Biochem Pharmacol* **12**: 539–550, 1963.
28. Frimmer M and Ziegler K, The transport of bile acids in liver cells. *Biochem Biophys Acta* **947**: 75–99, 1988.
29. Inoue M, Akerboom TPM, Sies H, Thao T and Arias IM, Biliary transport of glutathione *S*-conjugate by rat liver canalicular membrane vesicles. *J Biol Chem* **259**: 4998–5002, 1984.