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HEPATOBILIARY ELIMINATION OF THE PEROXISOME PROLIFERATOR NAFENOPIN BY CONJUGATION AND SUBSEQUENT ATP-DEPENDENT TRANSPORT ACROSS THE CANALICULAR MEMBRANE

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Abstract—Amphiphilic carboxylates acting as peroxisome proliferators and hypolipidemic drugs induce enzymes of peroxisomal lipid β-oxidation, certain drug-metabolizing enzymes in the liver, and a number of additional proteins. The peroxisome proliferators represent a well-established class of non-genotoxic hepatocarcinogens. In this study we characterized the hepatic elimination of the peroxisome proliferator nafenopin. In the rat *in vivo*, 1 hr after intravenous administration of [^{3}H]nafenopin, approx. 40% of injected radioactivity was recovered in bile. HPLC analysis of bile samples revealed that only about 10% of the radioactivity recovered in bile was associated with non-metabolized nafenopin and approx. 90% with more polar metabolites. One of the main metabolites formed in the liver and excreted into bile was identified as nafenopin glucuronide by β-glucuronidase-catalysed reconversion to nafenopin. In mutant rats deficient in the canalicular transport of leukotriene C_4 and related amphiphilic anion conjugates, recovery of [^{3}H]nafenopin-derived radioactivity in bile was reduced to 4% of the injected dose. Although nafenopin glucuronide could not be detected in bile, it was a major metabolite in the liver from these mutant rats. Using membrane vesicles enriched in bile canalicular membranes from normal rats, transport of nafenopin glucuronide was shown to be a primary-active ATP-dependent process which was inhibited by leukotriene C_4 and *S*-dinitrophenyl glutathione with IC_{50} values of 0.2 and 12 μM, respectively. ATP-dependent transport was not detectable for non-conjugated nafenopin. In canalicular membrane vesicles prepared from the mutant rats, the rate of ATP-dependent transport of nafenopin glucuronide was less than 10% of the transport observed in vesicles from normal rats. These data indicate that conjugation and subsequent transport by the ATP-dependent export carrier for leukotriene C_4 and related conjugates is a major pathway for the elimination of nafenopin and structurally-related peroxisome proliferators.

Key words: ATP-dependent transport; bile canalicular membrane; leukotriene C_4; nafenopin; nafenopin glucuronide; peroxisome proliferator

Many xenobiotic amphiphilic carboxylates used as hypolipidemic drugs, such as clofibrate and Naf§, induce peroxisome proliferation and in long-term studies hepatocarcinogenesis in rodents [1, 2]. They are non-genotoxic carcinogens which probably act as tumor promotors by modulating the expression of genes involved in growth and differentiation [3]. The administration of peroxisome proliferators leads to a rapid induction of genes encoding enzymes of peroxisomal lipid β-oxidation as well as some non-peroxisomal activities [4, 5]. These include a limited number of isoforms of drug-metabolizing enzymes, such as cytochrome P450 IVA1, cytosolic epoxide hydrolase and the glucuronosyltransferase which glucuronidates bilirubin, as well as liver thyroid hormone-related genes and adipose conversion [5–10]. The transcriptional induction by these compounds is mediated by PPARs, which are members of the steroid hormone receptor superfamily [11–14].

The liver is the main target organ of peroxisome proliferator action [5]. It is also the main site for inactivation and excretion of a variety of endo- and xenobiotics. Conjugation with glucuronate catalysed by glucuronosyltransferase (EC 2.4.1.17) plays an important role in the metabolism of several endogenous compounds such as bilirubin [15] and is one of the most important phase II reactions in drug metabolism [16]. Glucuronidation of the carboxyl moiety was observed for clofibrate and structural analogs in liver and kidney of several species [8, 17, 18].

Primary-active ATP-dependent transport is a

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§ Abbreviations: AdoPP[CH_2]P, adenosine 5′-[β,γ-methylene]-triphosphate; DNP-SG, *S*-(2,4-dinitrophenyl)-glutathione; GlcA, glucuronate; HTMP, 4-hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl; IC_{50}, concentration required for 50% inhibition; LTC_4, leukotriene C_4; Naf, nafenopin, 2-methyl-2-[*p*-(1,2,3,4-tetrahydro-1-naphthyl)-phenoxy]propionate; Naf-GlcA, nafenopin glucuronide; PPAR, peroxisome proliferator-activated receptor; TR$^-$ rats, Wistar rats deficient in the canalicular transport of leukotriene C_4 and related conjugates [29–31]; UDPGlcA, uridine 5′-diphosphoglucuronate; GSH, reduced glutathione.

major mechanism to excrete endogenous and exogenous substances across the hepatocyte canalicular membrane into bile. At least four distinct ATP-dependent carriers are localized in this membrane domain: (i) the *mdr 1* gene product (P-glycoprotein) which mediates the export of daunorubicin and related hydrophobic, cationic drugs [19–21]; (ii) the *mdr 2* gene product which seems to mediate the transport of phospholipids into bile [22], (iii) the ATP-dependent bile salt export carrier [23–25]; and (iv) the ATP-dependent leukotriene export carrier, which transports the glutathione conjugate LTC_4 with the highest affinity, and additionally, a variety of structurally related conjugates [26–28]. This transport system is deficient in a mutant rat strain (TR^-) lacking the capacity to secrete leukotrienes into bile [29]. These mutants are partially deficient in the hepatobiliary excretion of several other non-bile salt amphiphilic organic anions, such as bilirubin glucuronide and dibromosulfophthalein [30, 31].

In the present study the mechanism of hepatic elimination of Naf was studied in normal and TR^- mutant rats *in vivo* as well as *in vitro* by transport assays in bile canalicular membrane vesicles.

MATERIALS AND METHODS

Materials. [U-^{3}H]Naf (830 GBq/mmol) was obtained by custom service tritiation (Nuclear Research Center, Negev, Israel) of Naf (kindly donated by Ciba-Geigy). The purity of the labeled product was determined by silicic acid thin-layer chromatography in xylene/butan-2-one/acetic acid (50:50:3, by vol.) and RP-18 thin-layer chromatography in acetic acid/acetonitrile/water (11:6:3, by vol.). LTC_4 was obtained from Amersham-Buchler (Braunschweig, F.R.G.). DNP-SG was prepared from GSH and 1-chloro-2,4-dinitrobenzene using basic glutathione *S*-transferases from rat liver [32, 33]. ATP, creatine phosphate, UDPGlcA and glucuronosyltransferase (EC 2.4.1.17) from rat liver microsomes were obtained from the Sigma Chemical Co. (St Louis, MO, U.S.A.). Creatinine kinase, β-glucuronidase/arylsulfatase (EC 3.2.1.31/ EC 3.1.6.1) from *Helix pomatia* and β-glucuronidase from *Escherichia coli* were from Boehringer Mannheim (Mannheim, F.R.G.). Nitrocellulose filters (pore size 0.2 μm) were from Schleicher & Schüll (Dassel, F.R.G.) and scintillation fluids were from Canberra Packard (Warrenville, IL, U.S.A.).

Animals. For *in vitro* as well as *in vivo* studies, male Wistar rats (150–300 g) were obtained from the Zentralinstitut für Versuchstierzucht (Hannover, F.R.G.). Male Groningen Yellow (GY or TR^- rats) [30, 31] were provided by Dr F. Kuipers (Department of Pediatrics, University of Groningen, Groningen, The Netherlands). Animals were maintained on a standard diet with free access to food and water.

In vivo *elimination of* [^{3}H]*Naf.* Rats were anesthetized by intraperitoneal injection of ketamine (80 mg/kg body weight) and xylazine (12 mg/kg). Cannulation of the common bile duct and of the superior vena cava through the jugular vein were performed while rats were under general anesthesia as described [29]. The experiments were started by intravenous injection of [^{3}H]Naf (740 kBq/kg body weight; 0.9 nmol/kg) dissolved in 0.9% saline. Blood samples were withdrawn from the jugular catheter at 2, 5, 10, 20 and 60 min after [^{3}H]Naf injection. Bile and urine were collected into ice-cold 90% aqueous methanol containing 1 mM HTMP and 0.5 mM EDTA and aliquots were counted for radioactivity. One hour (or in some experiments 10 min) after [^{3}H]Naf injection the animals were killed by exsanguination. The livers were perfused *in situ* with 0.9% saline via the portal vein (to remove blood) and frozen in liquid nitrogen. The radioactivity in liver tissue and blood was determined in the supernatants of methanol extracts of the respective samples. Liver tissue (1 g) was disrupted in 15 mL of 100% methanol with an Ultraturrax mechanical homogenizer (Janke & Kunkel, Staufen, F.R.G.) and centrifuged at 15,000 *g* for 10 min at −10°. Samples of the methanol extract were dissolved in scintillation fluid and counted for radioactivity. Radioactivity in the whole blood was calculated assuming that the whole blood volume (mL) corresponds to 6.3% of the body weight (g) [34].

HPLC analysis. Separation of [^{3}H]Naf and its metabolites were performed by reversed-phase HPLC using a linear water/acetonitrile gradient. Precipitated proteins in samples containing 80% methanol were removed by centrifugation. The supernatants were evaporated to dryness, redissolved in 30% methanol and loaded on a C_{18} Hypersil column equilibrated with water containing 0.1% acetic acid adjusted to pH 5.7 with ammonium hydroxide. From 5 to 15 min a linear gradient was generated up to 38% acetonitrile followed by 30 min of 38% acetonitrile. The flow rate was 1 mL/min and the radioactivity in the eluate was determined continuously. Under these conditions standard [^{3}H]Naf was eluted in a single peak with a retention time of 45 min.

Synthesis and reconversion of [^{3}H]*Naf glucuronide.* Naf-GlcA was synthesized from [^{3}H]Naf and UDPGlcA using glucuronosyltransferase (EC 2.4.1.17) from rat liver microsomes [35]. [^{3}H]Naf was dissolved in 50 μL of 50 mM Tris–HCl pH 7.4 containing 10 mM $MgCl_2$, 10 mM UDPGlcA and 1.5 mU glucuronosyltransferase. After 40 min at 37° the incubation was terminated by addition of 150 μL methanol. Precipitated protein was removed and the supernatant subjected to HPLC separation. The HPLC fractions containing [^{3}H]Naf glucuronide were evaporated to dryness and redissolved in 90% ethanol. This ethanolic stock solution was checked again by HPLC and diluted in the incubation buffer (250 mM sucrose, 10 mM HEPES–Tris, pH 7.4) immediately before use in the transport assay.

Incubation of [^{3}H]Naf metabolites from liver and bile samples with β-glucuronidase/arylsulfatase from *Helix pomatia* and β-glucuronidase from *Escherichia coli* were performed in a buffer containing 0.1 M ammonium acetate pH 5.8 and pH 6.8, respectively [36]. After 30 min at 37° the incubations were stopped by addition of 4 vol. of 100% methanol and subjected to HPLC analysis.

Measurement of ATP-dependent [^{3}H]*Naf glucuronide transport into inside-out canalicular membrane vesicles.* Plasma membrane vesicles from rat

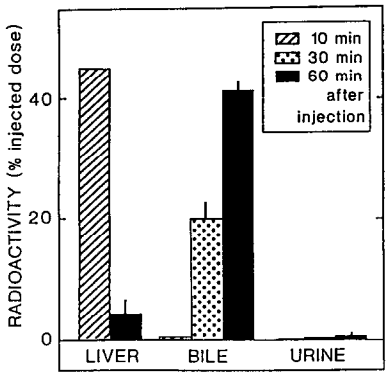

Fig. 1. Distribution of [^{3}H]Naf-derived radioactivity in normal rats. [^{3}H]Naf (740 kBq/kg body weight) was injected intravenously into bile duct-cannulated rats and [^{3}H]Naf-derived radioactivity determined in liver, bile and urine at the time points indicated and expressed as per cent of the injected dose. Data for 30 and 60 min after [^{3}H]Naf administration represent mean values from four animals (±SD); the data for 10 min after injection are from three animals with the deviation below 15%.

liver and fractions enriched in the hepatocyte canalicular domain were prepared and characterized as described [23, 26, 37]. Ultrastructural examination of the prepared membrane vesicles revealed predominantly closed vesicles of various size (mean diameter 0.5 μm) and few open membrane sheets [37]. Transport of [^{3}H]Naf glucuronide into the vesicles was measured by use of a rapid filtration technique [23, 26]. Membrane vesicles (30 μg of protein) were incubated in the presence of 4 mM ATP, 10 mM $MgCl_2$, 10 mM creatine phosphate, 100 μg/mL creatine kinase, and the labeled substrate, at the concentration indicated, in 250 mM sucrose, 10 mM HEPES–Tris, pH 7.4 (incubation buffer) [38]. GSH (5 mM) was added to the incubation medium to prevent a possible binding of the substrate to glutathione *S*-transferase. The final volume was 110 μL. Aliquots (20 μL) were taken at the time points indicated and diluted in 1 mL ice-cold incubation buffer. The diluted samples were filtered immediately through nitrocellulose filters (0.2 μm pore size), presoaked in incubation buffer by use of a rapid filtration device (Millipore Corp., Bedford, MA, U.S.A.) and rinsed twice with 5 mL of incubation buffer. Filters were dissolved and counted in a liquid scintillation counter. In control experiments, ATP was replaced by an equal concentration of 5′-AMP or, in some experiments, by AdoPP[CH_2]P. Uptake rates were calculated by subtracting the corresponding values in the presence of 5′-AMP or AdoPP[CH_2]P from those in the presence of ATP.

Statistical analysis. Data are given as mean values ± SD.

RESULTS

Elimination of [^{3}H]*Naf in normal and TR$^-$ rats* in vivo

The hepatobiliary excretion of [^{3}H]Naf and its metabolites was studied in normal rats and in rats deficient in the canalicular excretion of LTC_4 and related conjugates (TR$^-$ rats). In normal animals [^{3}H]Naf was preferentially taken up by the liver after intravenous administration and [^{3}H]Naf-derived radioactivity was subsequently excreted into bile (Fig. 1). When the experiments were terminated 10 min after [^{3}H]Naf injection, 45% (N = 3) of the injected radioactivity was found in the liver. One hour after administration 42 ± 1% (N = 4) of the injected radioactivity was recovered in bile.

HPLC analysis of the bile fractions revealed that only 10 ± 2% of the radioactivity in bile (corresponding to 4% of the injected radioactivity) was associated with non-metabolized Naf and approx. 90% with more polar metabolites (Fig. 2, middle panel). After HPLC separation the isolated metabolites from bile and liver extracts were incubated with β-glucuronidase/arylsulfatase from *Helix pomatia* and β-glucuronidase from *Escherichia coli* to identify conjugates with sulfate or glucuronate.

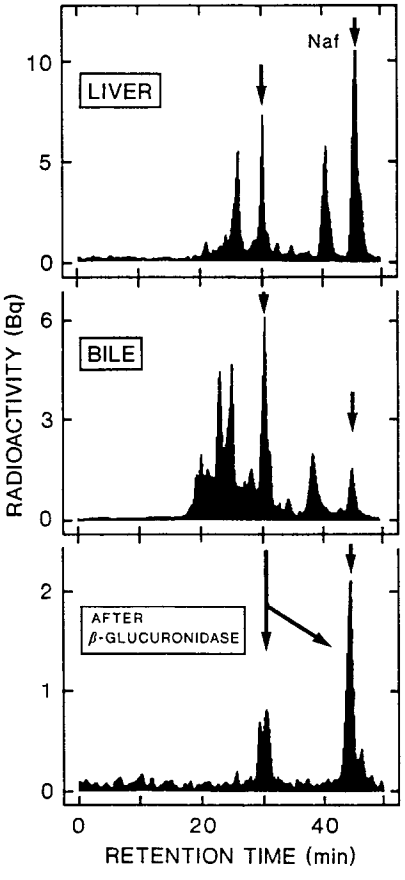

Fig. 2. [^{3}H]Naf metabolites in liver and bile of normal rats. Liver (upper panel) and bile (middle panel) 60 min after [^{3}H]Naf injection were analysed by reversed-phase HPLC as described in Materials and Methods. Standard [^{3}H]Naf was eluted with a retention time of 45 min. The isolated metabolite with the retention time of 30 min was reconverted to Naf by treatment with β-glucuronidase from *Escherichia coli* (lower panel).

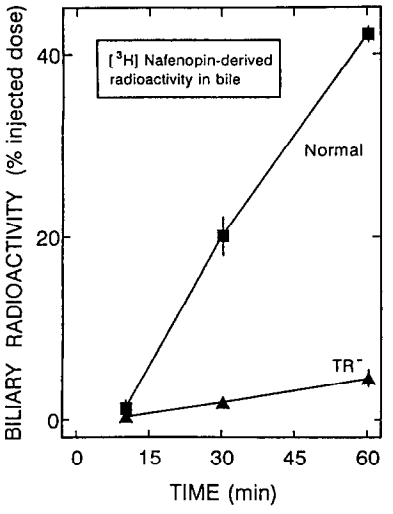

Fig. 3. Hepatobiliary excretion of [^{3}H]Naf-derived radioactivity in normal and TR$^-$ mutant rats. [^{3}H]Naf (740 kBq/kg body weight) was injected intravenously in normal rats (■) and rats deficient in the canalicular transport of LTC_4 and related conjugates (TR$^-$, ▲). Bile was sampled continuously and the radioactivity recovered from bile was expressed as per cent of the injected dose. Mean values ± SD from four animals.

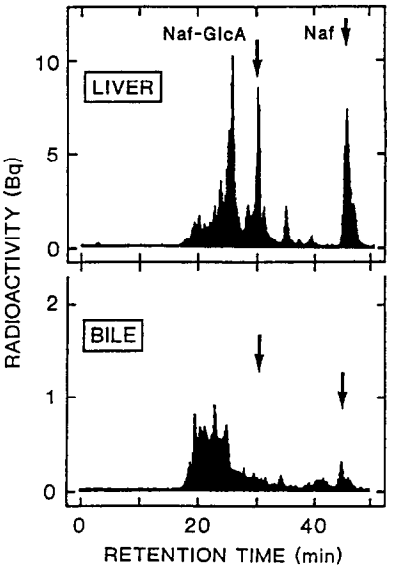

Fig. 4. [^{3}H]Naf metabolites in liver and bile of TR$^-$ mutant rats. Extracts from liver (upper panel) and bile (lower panel) of transport mutant rats 60 min after [^{3}H]Naf administration were analysed by reversed-phase HPLC. The retention time of synthetic Naf-GlcA and Naf standards are indicated by arrows.

One of the main metabolites with a retention time of 30 min, which was formed in the liver (Fig. 2, upper panel) and excreted into bile (Fig. 2, middle panel), was reconverted to Naf by incubation with β-glucuronidase/arylsulfatase as well as by the more specific β-glucuronidase enzyme from *Escherichia coli* (Fig. 2, lower panel). Furthermore, this metabolite co-chromatographed with synthetic Naf-GlcA. The polar metabolites with retention times between 20 and 30 min, also present in liver and bile, were not affected by β-glucuronidase/arylsulfatase treatment, indicating that they are neither glucuronides nor sulfate conjugates.

In TR$^-$ rats the recovery of [^{3}H]Naf-derived radioactivity in bile 1 hr after administration was reduced to 4.5 ± 1.0% of the injected dose (N = 4), corresponding to 11% of the recovery in normal rats (Fig. 3). The basal bile flow in these mutant rats was reduced to approx. 45% of that observed in normal rats (32 ± 2 vs 72 ± 13 μL/min × kg body weight). [^{3}H]Naf elimination from blood was not significantly different for the two groups. In normal rats 52 ± 3% of injected radioactivity was found in blood 2 min after [^{3}H]Naf injection and 16 ± 1% after 10 min. The corresponding values in the blood of TR$^-$ rats were 53 ± 2 and 14 ± 1% after 2 and 10 min, respectively. In urine collected over 60 min after injection, 1.5 ± 0.6% of the injected dose was found in TR$^-$ rats and 0.7 ± 0.3% in normal rats. The radioactivity remaining in the liver of TR$^-$ rats 60 min after administration was 18 ± 4% of the injected dose as compared with 4 ± 2% (N = 4) recovered from the liver of normal rats.

HPLC analysis of the bile fractions and liver extracts from TR$^-$ rats (Fig. 4) revealed that Naf-GlcA (retention time 30 min) was one of the major metabolites in the liver (Fig. 4, upper panel) from these mutant rats, but was not detected in their bile (Fig. 4, lower panel). The recovery of the polar metabolites with retention times between 20 and 30 min was also reduced in the bile of the TR$^-$ rats.

ATP-dependent transport of [^{3}H]*Naf glucuronide into isolated canalicular membrane vesicles.*

[^{3}H]Naf-GlcA was synthesized and purified as described in Materials and Methods. Figure 5 (left panel) shows the time course of transport of [^{3}H]-Naf-GlcA into canalicular membrane vesicles prepared from normal rat liver. This transport was stimulated by ATP. For a better differentiation between nucleotide-dependent binding and ATP-dependent transport into the vesicles, the rate of ATP-dependent transport was calculated from the difference in transport in the presence of 4 mM ATP and 4 mM 5′-AMP. Subtraction of the AMP blank values from the transport values in the presence of ATP resulted in a linear curve within the first 4 min of incubation. Initial rates of transport were calculated from the slope of this curve and amounted to 0.2 pmol/mg protein × min at a substrate concentration of 100 nM and to 0.47 pmol/mg protein × min at a substrate concentration of 250 nM [^{3}H]Naf-GlcA. As an additional control, the non-hydrolysable ATP-analog AdoPP[CH_2]P was used. These control values were not significantly different from those calculated in the presence of 5′-AMP (not shown). ATP-dependent transport was

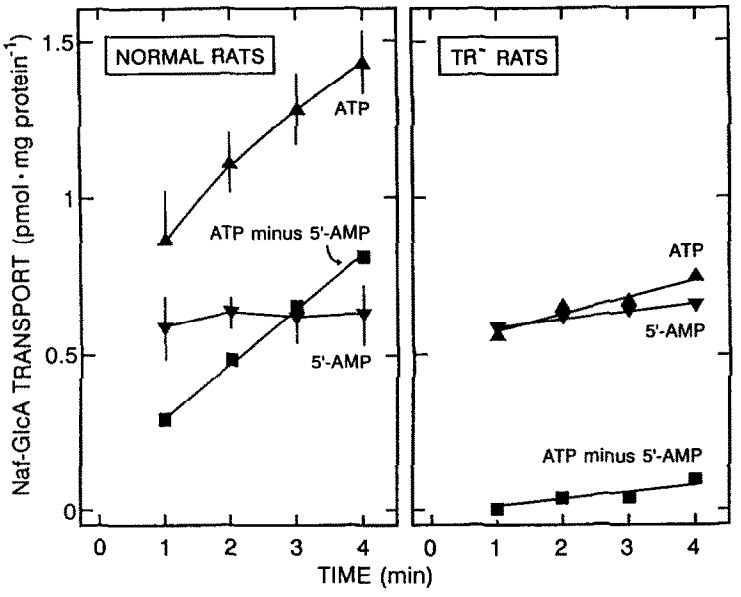

Fig. 5. ATP-dependent transport of [^{3}H]Naf glucuronide into canalicular membrane vesicles. Vesicles of bile canalicular membranes (30 μg of protein) from normal (left panel) and TR$^-$ mutant rats (right panel) were incubated with [^{3}H]Naf glucuronide (100 nM) and vesicle-associated radioactivity was determined by a rapid filtration technique as described in Materials and Methods. The rate of ATP-dependent transport (■), which proceeds into the inside-out oriented vesicles, was calculated from the difference in transport in the presence of 4 mM ATP (▲) or 4 mM 5'-AMP (▼). Mean values from four experiments in normal rats (±SD) and from three experiments in TR$^-$ rats.

detectable for non-conjugated Naf. Within the incubation time (4 min) no significant hydrolysis of Naf glucuronide was observed.

Canalicular membrane vesicles were also prepared from TR$^-$ rats. The rate of ATP-dependent transport into these vesicles was less than 10% of the rate observed in membranes from normal rats (Fig. 5, right panel). ATP-dependent transport of [^{3}H]Naf-GlcA into canalicular membrane vesicles from normal rats was inhibited by LTC_4 and DNP-SG with half-maximal inhibition at 0.2 and 12 μM, respectively (Fig. 6).

DISCUSSION

The main conclusions from this investigation can be summarized as follows: (i) Naf-GlcA is one of the main metabolites of Naf in the liver of normal as well as transport mutant (TR$^-$) rats and is excreted into the bile of normal but not the bile of TR$^-$ rats (Figs 2 and 4); (ii) in TR$^-$ rats the excretion of Naf metabolites into bile is decreased to approx. one tenth of that in normal rats (Fig. 3), whereas uptake into the liver is not affected as indicated by a similar elimination from the blood and higher recovery in the liver (18% of the injected dose after 1 hr compared with 4% in normal rats); (iii) and the transport of Naf-GlcA, but not of native Naf, across the bile canalicular membrane of normal rat liver is an ATP-dependent process, and can be inhibited by LTC_4 and other glutathione conjugates (Figs 5 and 6).

Peroxisome proliferators have been recognized as

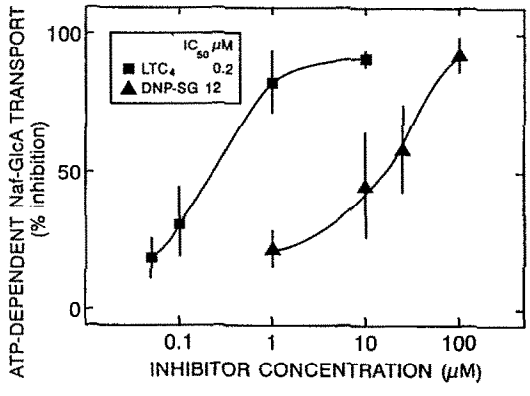

Fig. 6. Inhibition of ATP-dependent transport of [^{3}H]Naf glucuronide into canalicular membrane vesicles from normal rats by LTC_4 (■) and *S*-dinitrophenyl glutathione (▲). Membrane vesicles (30 μg of protein) were incubated with 100 nM [^{3}H]Naf glucuronide in the presence of the inhibitors, at the concentrations indicated, for 2 min at 37°. Initial rates of ATP-dependent transport were calculated from the slope of the ATP minus AMP curve as shown in Fig. 5. Data for each inhibitor concentration represent percentage inhibition of transport as compared to controls in the absence of the inhibitors (mean values ± SD, N = 4). IC_{50} values were defined as the concentrations required for 50% inhibition as compared to control values.

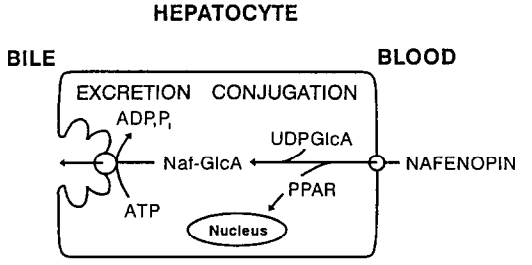

Fig. 7. Processes involved in the action and elimination of Naf in hepatocytes. Induction of specific enzymes by Naf is mediated by activation of a PPAR. In addition, activation of Naf to its coenzyme A ester and subsequent acylation of liver proteins has been described [39]. Hepatobiliary elimination of Naf is attained by conjugation with glucuronate, transferred from UDPGlcA and the subsequent ATP-dependent transport of Naf-GlcA by the export carrier for LTC_4 and related conjugates across the canalicular membrane into bile.

an interesting class of non-genotoxic chemical hepatocarcinogens. Signal transduction by these compounds is mediated by interaction with specific receptor proteins belonging to the steroid hormone receptor superfamily of ligand-activated transcription factors (Fig. 7) [11–14]. Their active concentration in the hepatocytes is determined by the rate of their inactivation and elimination. Peroxisome proliferators that possess a carboxyl group have been shown to be good substrates for glucuronosyltransferase [8, 17, 18]. In human urine, approx. 25% of the administered dose of clofibrate is recovered within 8 hr, predominantly as glucuronide [18]. In our study urinary recovery in the rat 1 hr after intravenous injection was relatively low in normal as well as in TR^- rats (<3%; Fig. 1). This may be due to the short experimental period, to the species difference and to the higher molecular weight of nafenopin as compared to clofibrate. Peroxisome proliferators, especially aryloxycarboxylates such as clofibrate and its analogs, are able to enhance the expression of the glucuronosyltransferase isoform involved in the conjugation of bilirubin. There is evidence, however, that this isoenzyme does not catalyse the glucuronidation of the peroxisome proliferators tested so far, in spite of the fact that both types of substrate form acylglucuronides [8].

Peroxisome proliferators are also activated to acyl coenzyme A esters, which are presumably involved in the acylation of liver proteins [39]. However, the amount of acylation is negligible as compared with the efflux by conjugation and subsequent transport into bile, as demonstrated by the fact that Naf-derived radioactivity accumulates predominantly in bile (Fig. 1). Covalent binding of Naf glucuronide to membrane proteins, however, can contribute to the ATP-*independent* binding of this compound to the membrane vesicles in our transport assay.

ATP-dependent transport of glutathione *S*-conjugates was originally described in erythrocyte inside-out membrane vesicles [40] and subsequently observed in several other tissues [26, 27, 33, 41–43]. In the liver it was recognized as an important transport system excreting several non-bile salt amphiphilic organic anions, including bilirubin conjugates and cysteinyl leukotrienes as endogenous substrates, into bile [26, 44]. The absence or inactivity of this exporter in the TR^- rat strain is considered to be analogous to the defect in the Dubin–Johnson syndrome in man [30, 45]. LTC_4 is the endogenous substrate with the highest known affinity for this export carrier [26, 28]. ATP-dependent transport of *p*-nitrophenyl glucuronide in canalicular membranes, which was inhibited by *S*-dinitrophenyl glutathione, has also been demonstrated [46]. The transport rates observed with Naf-GlcA in the present study were approx. two orders of magnitude lower than those observed with LTC_4 under similar conditions [26, 38].

Peroxisome proliferator treatment has been found to be associated with an inhibition of hepatic glutathione *S*-transferase-mediated bromosulfophthalein excretion in several studies [47–49]. This may be due to glutathione *S*-transferase inhibition [47, 50], but also to competition of the glucuronidated peroxisome proliferator and conjugated bromosulfophthalein at the ATP-dependent canalicular conjugate transporter. The excretion of other substrates of this export system can also be altered during peroxisome proliferator treatment. It remains to be investigated whether the Naf glucuronide excreted into bile undergoes, in part, enterohepatic circulation, which may involve hydrolysis by bacterial β-glucuronidases.

The results of the present study indicate that glucuronidation followed by ATP-dependent transport of the glucuronide across the canalicular membrane is an important pathway for the elimination of Naf and probably of other aryloxycarboxylates by the liver (Fig. 7). This ATP-dependent transport is mediated by the canalicular leukotriene export carrier, as indicated by the strong inhibition by LTC_4 and the impaired hepatobiliary elimination in transport mutant rats.

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