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Original article

The applicability of rat and human liver slices to the study of mechanisms of hepatic drug uptake

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Abstract

In the present study we investigated the applicability of the liver slice model to study mechanisms of drug uptake. Four model compounds were investigated that enter hepatocytes via entirely different membrane transport mechanisms. Rhodamine B (RB), which enters hepatocytes by passive diffusion, was homogeneously distributed throughout the rat liver slice (250 μm thickness) within 5 min, indicating that the penetration rate into the slice and the diffusion rate into the cells are rapid. In contrast, lucigenin (LU), which is taken up by hepatocytes through adsorptive endocytosis, was detected in the inner cell layers after 15 min. Digoxin uptake into the slice showed a temperature-dependent component and was stereoselectively inhibited by quinine, which is compatible with the involvement of a carrier-mediated uptake mechanism. The neo-glycoalbumin $Lactose_{27}$-Human Serum Albumin ($Lact_{27}$-HSA) and the negatively charged Succinylated-Human Serum Albumin (Suc-HSA) entered the slices and were taken up temperature-dependently into hepatocytes and endothelial cells, respectively. The liver slice preparation is a valuable tool to investigate the mechanisms of cellular uptake of drugs. Moreover, the precision-cut liver slices offer the unique possibility to study both hepatocyte and endothelial cell function in human and rat liver.

Keywords: Modified albumins; Rhodamine B; Lucigenin; Digoxin

1. Introduction

In 1923, tissue slices were introduced by Otto Warburg (1923) as an in vitro preparation to study organ function. Liver slices were occasionally used in the 1960s and early 1970s to study drug transport phenomena in the liver (Crawhall & Davis, 1971; Crawhall & Segal, 1968; Ertt-mann & Damm, 1975; Gigon, Nayak, & Schanker, 1969; Kupferberg & Schankl, 1968; Ryrfeldt, 1973a, 1973b; Schanker & Solomon, 1963; Solomon & Schanker, 1963; Tews & Harper, 1969). The slices were prepared using razor blades and/or the Stadie Riggs tissue slicer, but the reproducibility of the slicing was rather variable and highly dependent on the skills of the operator. The slices that were produced at that time had a thickness of about 0.5 mm. Later, Smith et al. (1986) showed that in slices of 0.5 mm thickness tissue necrosis occurred in the middle of the slice during incubation. Therefore, only a part of the cells in the slice will be really involved in the uptake process studied, while cells in the functional part of the slice may have been partly deficient of O_2 and nutrients. The presence of these necrotic cells may influence the functioning of the neighboring cells. Data obtained from these slices may not be valid. When the techniques to use isolated hepatocytes and isolated livers perfusions were introduced and optimized (Berry, Halls, & Grivell, 1992; Meijer, Keulemans, & Mulder, 1981), these became the preferred in vitro preparations for drug transport studies. However, the studies in isolated hepatocytes are hampered by the fact that the isolation procedures involve collagenase digestion for the

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disruption of cell to cell contacts. Clearly, this proteolytic enzyme may also damage plasma membranes and transport systems therein. In addition, distribution into different cells of the liver cannot be studied with isolated hepatocytes.

However, since the introduction of the Krumdieck slicer, which allowed the production of thin slices of reproducible thickness and minimal trauma to the tissue, the use of liver slices has increased steadily in biological research. Most research using liver slices is performed in the field of toxicology and metabolism of xenobiotics (Olinga, Meijer, Slooff, & Groothuis, 1998). Only a few studies have been published on transport of drugs in liver slices prepared by the Krumdieck slicer (Thompson, Davies, & Morris, 1993; Worboys, Bradbury, & Houston, 1997).

The present study should be seen as an explorative study to investigate the possibilities and limitations of the use of liver slices in drug transport studies. Slices from rat and human livers were used. To obtain more information about the influence of the lipophilicity of drugs on the penetration into and diffusion within the slices, we used two fluorescent probes: rhodamine B (RB), a very lipophilic compound, which has been shown to enter liver cells by passive diffusion only (Braakman, Groothuis, & Meijer, 1987); and lucigenin (LU), a hydrophilic compound that is taken up by adsorptive endocytosis (Braakman et al., 1989).

To elucidate if very specific uptake mechanisms, described in other liver preparations, can be investigated using liver slices, we studied the stereoselective inhibition of the uptake of digoxin by quinidine and quinine in rat liver slices. In experiments with rat hepatocytes a marked inhibition of digoxin uptake by quinine and only minimal inhibition by the diastereomer quinidine was demonstrated (Hedman & Meijer, 1998).

In the present study, modified proteins were used to investigate if these relatively large molecules can enter both rat and human liver slices. The lactosylated protein $Lactose_{27}$-Human Serum Albumin ($Lact_{27}$-HSA), containing 27 lactose moieties per protein molecule, was previously shown to be taken up by endocytosis via the asialoglycoprotein receptor on the rat hepatocyte plasma membrane (van-der-Sluijs, Bootsma, Postema, Moolenaar, & Meijer, 1986).

To study the uptake function of endothelial cells we studied the uptake of two negatively charged proteins, Succinylated-Human Serum Albumin (Suc-HSA) and Aconylated-Human Serum Albumin (Aco-HSA). Suc-HSA and Aco-HSA are both taken up in the rat liver via receptor-mediated endocytosis, possibly by scavenger receptors mainly on the endothelial cell (Jansen, Olinga, Harms, & Meijer, 1993). To visualize the uptake of negatively charged proteins in endothelial cells in rat liver slices, FITC-Aco-HSA was used, a model compound that can be detected immunohistochemically. These different uptake processes were studied to examine the potential of using liver slices in drug transport research.

2. Materials and methods

2.1. Materials

The following compounds were obtained from the indicated sources: bovine serum albumin (BSA) from Organon Teknika (Boxtel, The Netherlands); University of Wisconsin organ preservation solution (UW) from Du Pont Critical Care (Waukegan, IL, USA). LU from Molecular Probes (Eugene, OR, USA). RB, *N*-acetyl-galactosamine (GalNac), quinine hydrochloride and quinidine hydrochloride mono-hydrate from Sigma (St. Louis, MO, USA); [^{3}H(G)]Digoxin (specific activity 15–30 Ci/mmol) from Du Pont NEN Research Products (Boston, USA). Nonradioactive digoxin from Boehringer Mannheim (Mannheim, Germany). Hionic fluor and Soluene 350 were obtained from Packard (Groningen, The Netherlands). Suc-HSA, $Lact_{27}$-HSA and Aco-HSA were prepared in our lab, as previously described (Jansen, Schols, Pauwels, De-Clerq, & Meijer, 1993; van-der-Sluijs et al., 1986). ^{125}I-Suc-HSA and ^{125}I-$Lact_{27}$-HSA were labeled as described by Jansen, Olinga, et al. (1993). FITC-Aco-HSA was prepared as described by Swart et al. (1996). The 24- and 6-well tissue culture plates were obtained from Costar (Cambridge, MA, USA). Tissue-tek from Miles (Elkhart, IN, USA). All other chemicals were of analytical grade and were obtained from commercial sources.

2.1.1. Human liver tissue

Human liver tissue was obtained from livers procured from multiorgan donors (Tx-livers) or from patients after partial hepatectomy because of metastases of colorectal carcinoma (PH-livers). Consent from the legal authorities and from the families concerned was obtained for the explantation of organs for transplantation purposes. The donor livers were reduced in order to perform reduced size or split liver transplantation. The donor liver was perfused with cold UW organ storage solution in situ before explantation. The livers were stored in cold UW solution on ice until reduction of the liver. The reduction or splitting of the donor organ was performed while immersed in UW cooled with ice slush. The liver tissue remaining after bipartition was stored again in cold UW solution until the start of the slicing procedure. Total cold preservation time varied from 6–39 h. The research protocols were approved by the medical ethical committee of our institution (University of Groningen). In case of the PH-livers consent from the patients concerned was obtained for the use of liver tissue for research purposes. The research protocols were approved by the medical ethical committee of our institution (University of Groningen). The technique of partial hepatectomy was performed as described earlier (Brouwers et al., 1997). After partial hepatectomy a wedge from the resected liver lobe was cut at distance from the metastases. Warm ischemia time in PH-livers defined as the time after clamping of the branches of the hepatic artery and portal vein until resection/excision, varied from 5 to 90 min. Directly after excision of

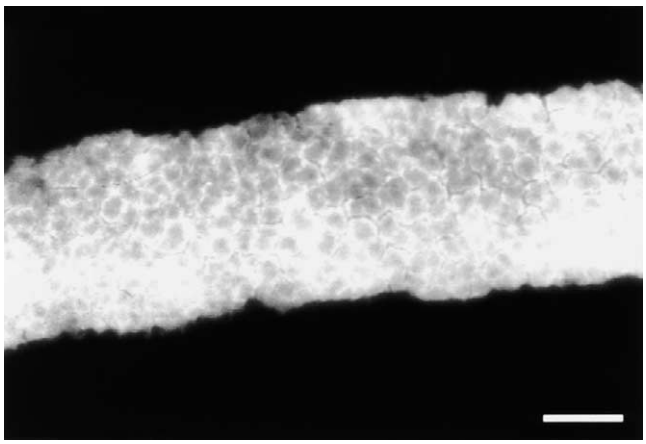

Fig. 1. The 25-μM RB distribution in the cross-section of the rat liver slice (~250 μm) after 5 min of incubation. (fluorescence microscopy, bar = 100 μm).

the piece of tissue the biopsy wedge was perfused with cold UW and was transported to the laboratory where the slicing procedure was started within 30 min.

2.1.2. Rat liver tissue

Male Wistar rats of 280–320 g were used in the experiment. Animals were anaesthetized by pentobarbital (ip 60 mg/kg), the livers were excised and handled as described in Preparation of liver slices.

2.1.3. Preparation of liver slices

Cores of approximately 8 mm were prepared from the pieces of liver tissue by advancing a sharp rotating metal tube in the liver tissue, and were subsequently placed in the Krumdieck slicer. The slicing was performed in ice-cold Krebs–Henseleit buffer (KHB) (Sandker, Slooff, & Groothuis, 1992) supplemented with glucose to a final concentration of 25 mM, saturated with 95% O_2 and 5% CO_2 and a pH of 7.42. Human and rat liver slices of about 200–300 μm thickness and a wet weight of 10–14 mg

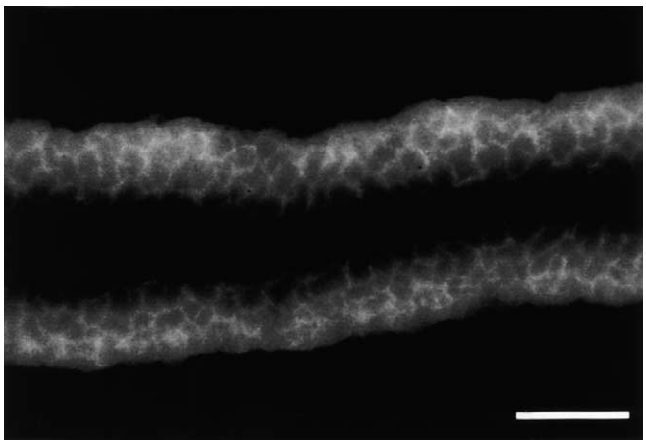

Fig. 2. The 25-μM RB distribution in the cross-section of the rat liver slice (~250 μm) after 3 min of incubation. (fluorescence microscopy, bar = 100 μm).

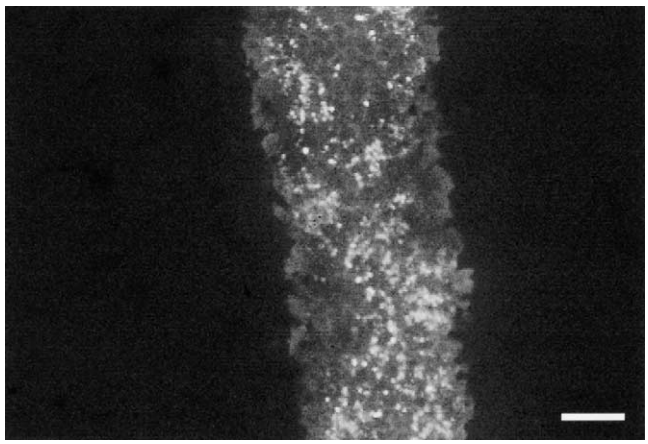

Fig. 3. The 5-μM LU distribution in the cross-section of the rat liver slice (~250 μm) after 90 min of incubation. (fluorescence microscopy, bar = 100 μm).

were prepared with the standard settings (cycle speed 40; interrupted mode) of the Krumdieck slicer. After slicing the human and rat liver slices were stored in ice-cold UW until the start of the experiment within an hour (Olinga, Merema, et al., 1998).

2.1.4. Incubation of liver slices

Human and rat liver slices were incubated in 1.4 ml KHB supplemented to 25 mM glucose +1% BSA on stainless steel grids in 24-well tissue culture plates at 37°C with separate magnetic stirrers and carbogen supply in each well (Olinga, Merema et al., 1998). Alternatively, slices were incubated in 6-well tissue culture plates in 3.2 ml KHB supplemented with 25 mM glucose and 1% BSA (Olinga

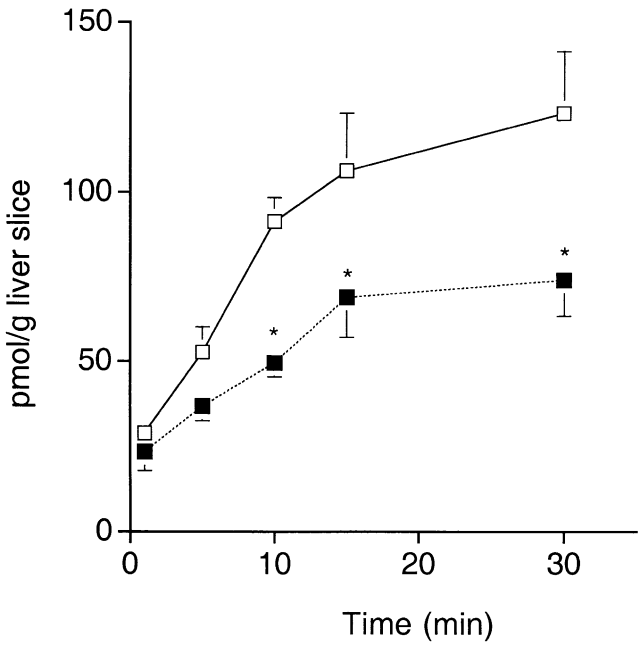

Fig. 4. The uptake of 25 nM digoxin at 37°C (□) and 4°C (■) in rat liver slices. Points are the mean of three separate experiments ± S.E.M. * $P < .05$.

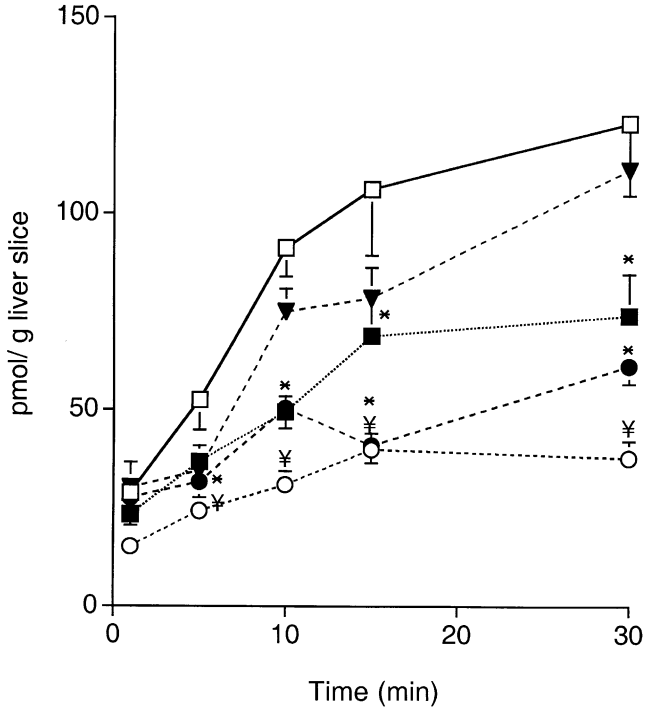

Fig. 5. The uptake of 25 nM digoxin at 37°C (□) and 4°C (■) in the presence of 50 μM quinidine at 37°C (●) and 4°C (○) or 50 μM quinine at 37°C (▼) in rat liver slices. Points are the mean of three separate experiments ± S.E.M. * $P < .05$ versus uptake of digoxin at 37°C. ¥ $P < .05$ versus uptake of digoxin at 4°C.

et al., 1997). These 6-well plates were placed in a plastic container that was continuously gassed with 95% O_2/5% CO_2 and incubated in a water bath at 42°C in order to keep the temperature of the medium at 37°C. The plastic container was shaken back and forth (110 times/min); at this frequency, optimal mixing was obtained and the slices moved freely in the medium without touching the walls (Olinga et al., 1997). No differences were found for the different incubation methods for incubations up to 3 h (Olinga et al., 1997).

2.1.5. Uptake experiments

2.1.5.1. Disposition of fluorescence dyes

The uptake of RB and LU were determined in the 6-well incubation system. Rat liver slices were preincubated for 1.5 h in the incubation system and the substrates were added in a final concentration of 25 μM for RB and 5 μM for LU. After regular intervals, the slices were taken out of the incubation system, washed in ice-cold KHB, completely embedded in Tissue-tek and snap-frozen in iso-

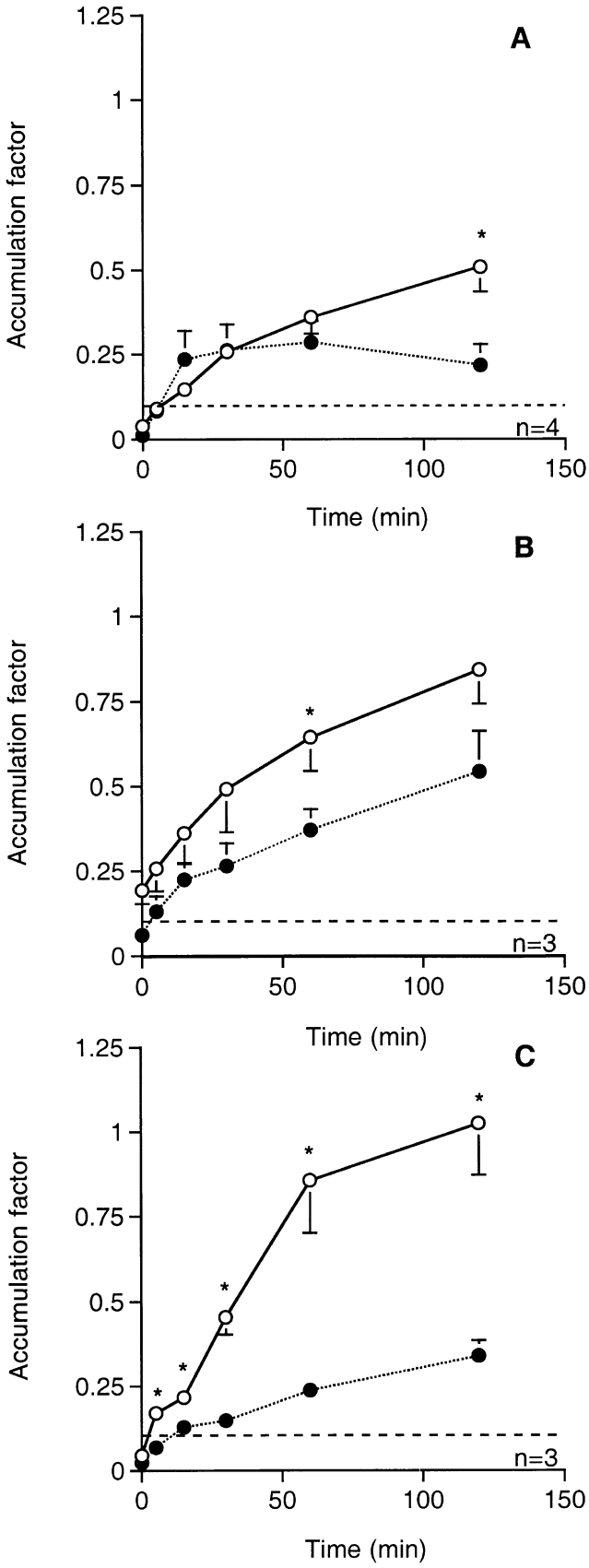

Fig. 6. Uptake of ^{125}I-Lact$_{27}$-HSA in liver slices from Tx-livers (A), from PH-livers (B) and rat livers (C) at 37°C (○) and 4°C (●). Points are the mean of separate experiments ± S.E.M. n = number of livers. * $P < .05$ versus 4°C. The dashed line is the accumulation factor if ^{125}I-Lact$_{27}$-HSA is exclusively distributed within the sinusoids.

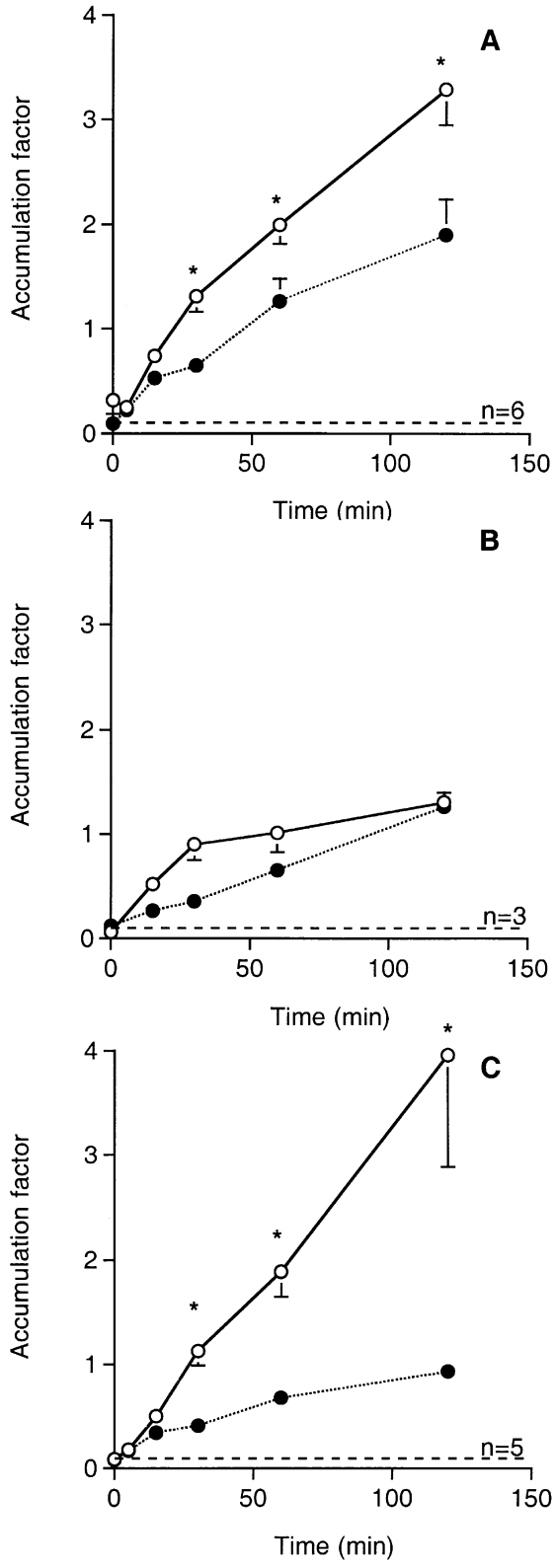

Fig. 7. Uptake of ^{125}I-Suc-HSA in liver slices from Tx-livers (A), from PH-livers (B) and rat livers (C) at 37°C (○) and 4°C (●). Points are the mean of separate experiments ± S.E.M. n = number of livers. * $P < .05$ versus 4°C. The dashed line is the accumulation factor if ^{125}I-Suc-HSA is exclusively distributed within the sinusoids.

pentane (− 80°C). Care was taken that the slices were frozen within 30 s. They were stored at − 80°C until sections of 8 μm were cut in a cryostat (− 20°C) perpendicular to the surface of the slice. The cross-section was allowed to dry on a glass microscope slide and examined unmounted under a Leitz Orthoplan fluorescence microscope with the appropriate fluorescence filters (RB: excitation = 530 to 560 nm; emission > 580 nm; LU: 450–490 nm excitation, emission 515 nm).

2.1.5.2. Disposition of digoxin

Digoxin uptake and inhibition studies were performed in the 6-well incubation system in triplicate. After preincubation for 1.5 h, rat liver slices were transferred to fresh KHB supplemented with 25 mM glucose and 1% (w/v) BSA. At $t = 0$ [^{3}H]digoxin was added together with nonradioactive digoxin to a final concentration of 25 nM. Inhibition studies were performed in presence of 50 μM of quinidine or quinine, which were added at the same time as digoxin. These concentrations were also used in a study with rat hepatocytes (Hedman & Meijer, 1998). After different time periods the slices were taken out of the well, washed twice in ice-cold KHB, submerged in counting vials containing 0.43 ml soluene 350/isopropanol (1:2) and incubated for 30 min at 45°C. After cooling to room temperature, 0.22 ml H_2O_2 was slowly added. Subsequently, 3.85 ml of Hionic fluor was added and the radioactivity was counted in a Beckman LS 1701 Liquid Scintillation System.

2.1.5.3. Disposition of proteins

After 1.5 h preincubation of the rat and human liver slices, the slices were incubated in triplicate with ^{125}I-Suc-HSA and ^{125}I-Lact$_{27}$-HSA in the 24-well incubation system. The concentration used of ^{125}I-Suc-HSA or ^{125}I-Lact$_{27}$-HSA was 1.4 μg/ml, incubation was either at 4°C to detect binding or at 37°C to measure uptake. After various time points, slices were taken out of the incubation system and washed in ice-cold KHB and the radioactivity was counted in LKB-Multichannel γ-counter. Inhibition of the binding of ^{125}I-Lact$_{27}$-HSA to the receptor was studied at 4°C in the presence of an excess of unlabelled Lact$_{27}$-HSA or GalNac (from 21 μg/ml–21 ng/ml). In the case of ^{125}I-Suc-HSA an excess of unlabelled Suc-HSA or Aco-HSA (from 21 μg/ml–21 ng/ml) was used. To express the extent of accumulation of a compound in the liver slice, the accumulation factor was used. This is defined as the concentration of the compound in the slices divided by the concentration in the medium.

To study the cellular localization of the protein in the slices, 1 mg/ml FITC-Aco-HSA was added to rat liver slices in a 24-well tissue culture plate. After 2 h of incubation at 37°C the slice was taken out and handled as described before for RB and LU. FITC-Aco-HSA was determined by immunohistochemical staining, as described by Swart et al. (1996).

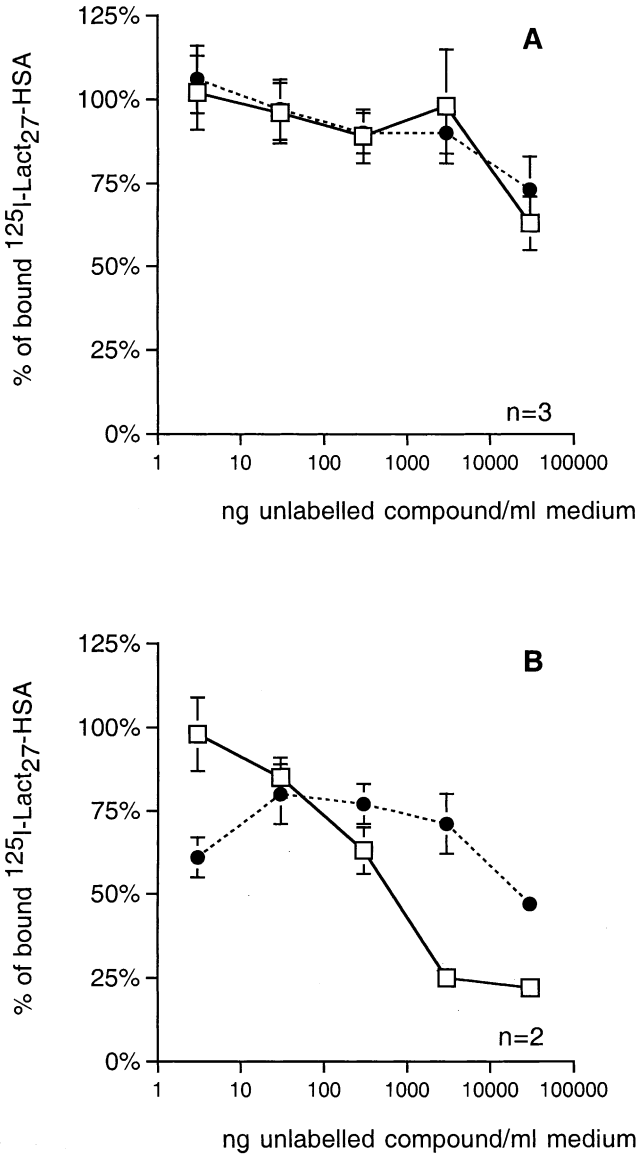

Fig. 8. Inhibition of binding at 4°C of ^{125}I-Lact$_{27}$-HSA by Lact$_{27}$-HSA (□) and GalNac (●) in human liver slices. n = number of livers.

2.1.6. Statistics

Results were compared using two-tailed Student's t test. A P value $< .05$ was considered significant.

3. Results

3.1. Disposition of fluorescence dyes

The cross-sections of slices incubated in the presence of RB showed a bright red fluorescence. At 37°C it took about 5 min before RB was distributed homogeneously throughout a slice of about 250–300 μm (Fig. 1). After 3 min only part of the liver slice contained the fluorescence (Fig. 2). After incubation at 4°C a similar distribution pattern was observed. After 90 min of incubation in the presence of 5 μM LU, fluorescence microscopy of the rat liver slice cross-sections revealed a patchy pattern, with large and small fluorescent spots, also in the middle of the slice (Fig. 3). Within 15–30 min the first spots were found in the middle of the slice and the number of spots increased in the subsequent 60 min. If the same experiment was performed at 4°C no fluorescence was found in the slice at all (data not shown).

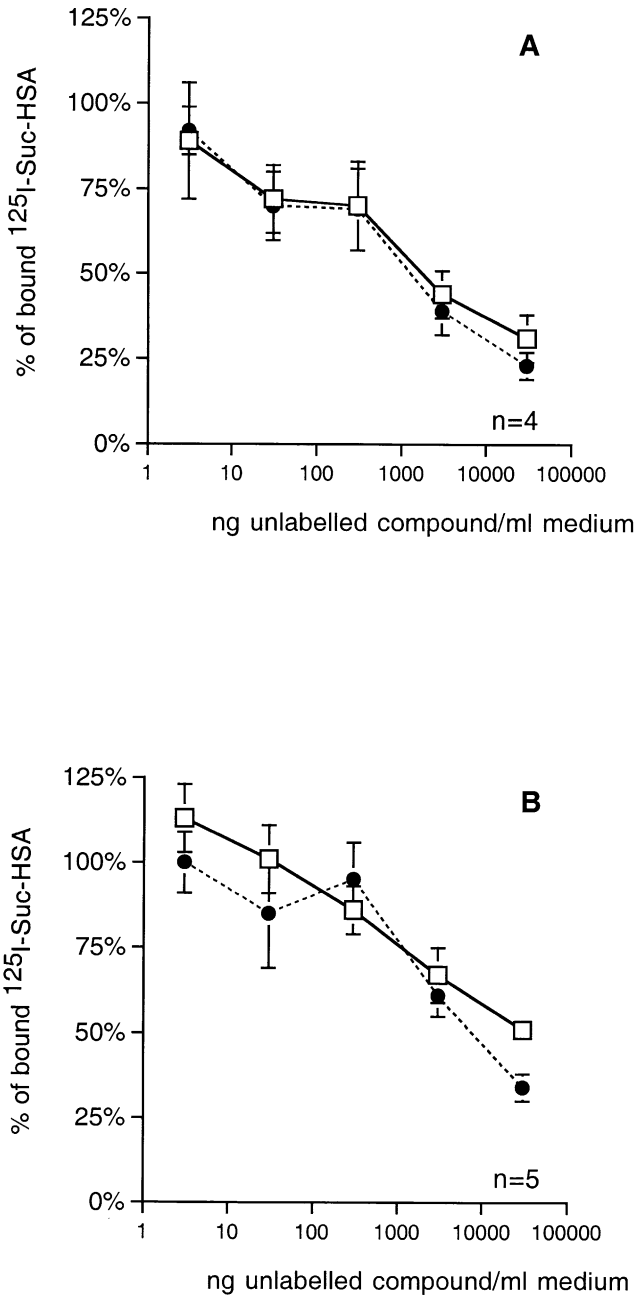

Fig. 9. Inhibition of binding at 4°C of ^{125}I-Suc-HSA by Suc-HSA (□) and Aco-HSA (●) in human liver slices (A) and rat liver slices (B). n = number of livers.

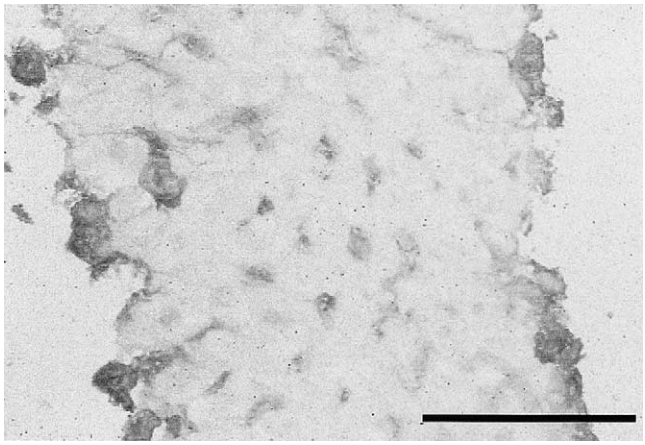

Fig. 10. FITC-Aco-HSA distribution in the cross-section of the rat liver slice (±250 μm) after 120 min incubation. (bar = 100 μm).

3.2. Disposition of digoxin

The uptake of 25 nM of digoxin at 37°C in rat liver slices was linear within the first 15 min of drug application. A clear difference could be found between the amount of digoxin taken up in the liver slice at 4°C and 37°C (Fig. 4). The accumulation ratio of digoxin in the slices after 30 min at 37°C was 4.9, while at 4°C the accumulation was 3.0. The uptake of 25 nM digoxin in rat liver slices was only slightly inhibited in the presence of 50 μM of quinidine. In contrast, 50 μM of quinine showed a clear inhibition (maximally 61% inhibition was observed) of the uptake of digoxin (Fig. 5). After incubation with digoxin at 4°C in the presence of 50 μM quinine, the amount of digoxin in the slice was lower than the digoxin amount in the slice at 4°C in the absence of 50 μM quinine (Fig. 5).

3.3. Disposition of proteins

The uptake of $Lact_{27}$-HSA in rat liver slices was temperature-dependent (Fig. 6, panel C). However, only partial temperature dependency was observed in the uptake of $Lact_{27}$-HSA for Tx-liver and PH-liver slices (Fig. 6, panels A and B).

The uptake of Suc-HSA in Tx-liver and rat liver slices had a temperature-dependent component (Fig. 7, panels A and C). For the human PH-liver slices no significant temperature-dependent uptake of Suc-HSA was observed. The liver slices prepared from Tx-livers showed a higher amount of Suc-HSA in the slice compared to PH-liver slices (Fig. 7, panels A and B).

Binding of ^{125}I-$Lact_{27}$-HSA in rat liver slices, was inhibited by increasing concentration of $Lact_{27}$-HSA (Fig. 8, panel B) at 4°C. GalNac inhibited the binding of ^{125}I-$Lact_{27}$-HSA to a lesser extent than $Lact_{27}$-HSA. Binding of ^{125}I-$Lact_{27}$-HSA in human liver slices from both human tissue sources was inhibited only significantly at the highest concentration of $Lact_{27}$-HSA and GalNac (Fig. 8, panel A). ^{125}I-Suc-HSA binding was inhibited by an excess of unlabelled Suc-HSA and Aco-HSA (Fig. 9, panels A and B) both in human and rat liver slices.

FITC-Aco-HSA was detected in all cell layers of the slice after 120 min of incubation (Fig. 10). The pattern revealed resemblance with a sinusoidal staining, as was previously described in rat liver (Jansen, Olinga, et al., 1993).

4. Discussion

4.1. Disposition of fluorescence dyes

RB is a very lipophilic compound and is very rapidly taken up by hepatocytes by passive diffusion (Braakman, 1988). In the present study it was fully distributed throughout the whole rat slice (of 250 μm) within 5 min. For tritium-labeled water it was also reported that it distributes all over the liver slice within 5 min (Worboys et al., 1997). It seems that a period of at least 5 min is necessary before a compound is homogeneously distributed completely within the presently used precision-cut slices. The uptake process of RB was not temperature dependent and no differences were found in slice uptake at 4 or 37°C. This is in line with the proposed mechanism of uptake that may comprise diffusion from the extracellular spaces into the lipid membranes (water/lipoid diffusion) and direct transfer of RB between neighboring cells through cell contacts (lipoid/lipoid diffusion) (Braakman, 1988).

In contrast to RB, LU is taken up by adsorptive endocytosis (Braakman et al., 1989), which is an energy requiring uptake process. The present results show that this endocytosis process is also operating in liver slices (Fig. 3). At 37°C there are sparkling fluorescence spots in the slice, indicating that this hydrophilic compound enters the cells and penetrates the slice as far as the inner cell layers within 15 min. At 4°C no fluorescence was found in the liver slices, indicating that cellular uptake and/or concentration in intracellular vesicles is necessary for the visualization of the dye and that an active uptake process is involved. Anyway, our results suggest a considerably slower rate of penetration of LU into the slice than of RB. This is probably not due to a limitation in detection because in perfused livers we observed LU-fluorescence already after 2 min (Braakman et al., unpublished results). Probably for this compound, diffusion through the aqueous phase of intra-slice-sinusoids determines the distribution rate within the slice.

4.2. Disposition of digoxin

The uptake of digoxin showed temperature dependency, indicating that the uptake is partly carrier-mediated, as was found in rat hepatocytes (Hedman & Meijer, 1998). After 30 min of incubation at 37°C digoxin was concentrated 4.9-fold in the slice, whereas after incubation at 4°C it was concentrated only 3.0-fold. The 3.0-fold accumulation at

4°C may be due to extracellular binding or intracellular binding after passive diffusion into the cells. The difference in accumulation between 4°C and 37°C indicates that temperature-dependent processes other than simple diffusion are functioning in the slice. Furthermore, a marked inhibition of the uptake of digoxin was found by quinine and only a minimal inhibition was found by the diastereomer quinidine. This stereoselective inhibition of digoxin was also observed in rat hepatocytes (Hedman & Meijer, 1998). The results indicate that quinine inhibits the carrier-mediated uptake completely, and as results at 4°C with quinine show, also inhibits the binding of digoxin. The uptake rate of digoxin in liver slices was found to be 0.1 pmol/10^6 hepatocytes/min (this study) and in isolated hepatocytes 0.75 pmol/10^6 hepatocytes/min using the same initial concentration (Hedman & Meijer, 1998). This may indicate that either the hepatocytes in the slice exhibit a much lower uptake rate or that only some of the cells in liver slices participate in the transport of the digoxin. The latter could be due to the relatively fast uptake of digoxin into the first layers of hepatocytes compared to the penetration rate into the sinusoids and the inner cell layers of the slice. The results with RB discussed above also show that in the first 5 min not all of the liver cells will be able to contribute to the uptake process. For the digoxin uptake rate measured in slices, the penetration into the slice may therefore be a rate-limiting step. Yet the results with digoxin indicate that functionality of drug carriers is maintained in liver slices, and that slices can be used to study the mechanisms and specificity of carrier-mediated uptake of drugs.

4.3. Disposition of proteins

The uptake of ^{125}I-Lact$_{27}$-HSA, a ligand for the asialoglycoprotein receptor (van-der-Sluijs et al., 1986), in rat liver slices resulted in a 1.7-fold accumulation of the ligand in the slice and exhibited a temperature-dependent component. This is a first clue for the involvement of a specialized uptake process, as was also found in rat hepatocytes (Jansen, Kruijt, Van Berker, & Meijer, 1993). At 4°C, binding to the receptor was inferred from the accumulation factor of 0.6. If the protein would have been exclusively distributed within the sinusoids, an accumulation factor of only 0.05–0.1 would have been found, assuming a sinusoidal volume of 5–10% of the tissue volume. Binding of ^{125}I-Lact$_{27}$-HSA was inhibited by increased concentrations of GalNac and Lact$_{27}$-HSA. This supports the idea that the uptake is a receptor-mediated mechanism, likely involving the asialoglycoprotein receptor. Importantly, the experiments show that receptor-mediated uptake of high molecular weight compounds such as neo-glycoproteins and charge-modified proteins up to 72 kDa is operative in the slice.

In human liver slices only the Tx-liver slices seemed to exhibit temperature-dependent uptake of ^{125}I-Lact$_{27}$-HSA after 2 h. This may indicate that Tx-livers have a better hepatocyte function than PH-liver slices. This was also found in a previous study comparing drug metabolism in Tx- and PH-livers (Olinga, Merema, et al., 1998). Quantitatively, the uptake of ^{125}I-Lact$_{27}$-HSA in human Tx- and rat liver slices is within the same range, compatible with results obtained in human and rat hepatocytes (Jansen, Kruijt, et al., 1993). In contrast, the results obtained with the uptake of low molecular weight substrates in isolated hepatocytes indicate that the uptake rate of vecuronium, ouabain and taurocholic acid is 5–10 fold lower in human than in rat hepatocytes (Sandker et al., 1994). The highest concentrations at 4°C of GalNac and Lact$_{27}$-HSA were able to inhibit the binding of ^{125}I-Lact$_{27}$-HSA in human liver slices. These results indicate that the uptake of Lact$_{27}$-HSA is regulated via receptor-mediated mechanism and that the asialoglycoprotein receptor is involved.

The temperature-dependent uptake patterns of ^{125}I-Suc-HSA in rat liver slices also indicate that a specialized uptake process is involved. An excess of unlabelled Suc-HSA and Aco-HSA, both substrates with high affinity for the scavenger receptor, inhibited the binding of ^{125}I-Suc-HSA in rat liver slices. This indicates the involvement of this receptor system in the uptake of ^{125}I-Suc-HSA.

After 120 min of incubation with FITC-Aco-HSA the inner cell layers in the rat liver slice showed clear sinusoidal staining. Collectively, this may indicate that endothelial cells are involved in the uptake of Aco-HSA and Suc-HSA and that the endothelial cells in the liver slice are functional in this respect. To the best of our knowledge, this is the first study showing endothelial cell function in liver slices.

In human liver slices, temperature-dependent uptake of ^{125}I-Suc-HSA was observed only in Tx-livers. Together with the fact that the binding of ^{125}I-Suc-HSA is much lower in PH-livers, this may indicate that endothelial cells in the Tx-livers are better preserved compared to the endothelial cells in the PH-livers. This observation can be explained by the fact that the warm ischemia, inevitable with the PH-livers, damages the endothelial cells. Endothelial cells play an important role in the damage occurring in liver transplantation during reperfusion period (Lemasters & Thurman, 1997). Currently, we are investigating whether the uptake rate of Suc-HSA may be used to assess the viability of the endothelial cells in the human liver.

In conclusion, liver slices seem to be a valuable tool to investigate the mechanisms of transport of drugs and drug targeting preparations. This is of special importance because this technique enables us to study these processes in human liver. Saturable and temperature-dependent drug uptake, stereoselective inhibition, and receptor-mediated uptake of proteins can be clearly demonstrated. Moreover, liver slices offer the unique possibility to study endothelial cell function in both animal and human liver. However, the uptake rate of compounds in the slice may not solely reflect the actual uptake rate of the cells involved, but also appears to be influenced by the rate of penetration into the slice. This process may be rate limiting in slice accumulation, if the cellular uptake is relatively fast.

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