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The Absence of Stereoselective P-Glycoprotein-mediated Transport of *R/S*-Verapamil Across the Rat Jejunum

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Abstract

We have studied the potential stereoselective transport and metabolism of *R/S*-verapamil in rat jejunum, in-situ.

A regional single-pass perfusion of the rat jejunum was performed on 24 rats in six separate groups. The effective permeability (P_{eff}) was assessed for three different concentrations of verapamil, 4, 40 and 400 mg L^{-1}. The P_{eff} of each enantiomer was also determined at 400 mg L^{-1} when chlorpromazine (10 mM) was added to the perfusion solution. Two other groups of rats received *R/S*-verapamil as an intravenous infusion and the intestinal secretion and metabolism were studied by simultaneously perfusing the jejunum with a control or with chlorpromazine (10 mM) added. The concentrations in the outlet perfusate of each enantiomer of verapamil and norverapamil were assayed with HPLC. *R/S*-Verapamil is a high permeability drug in the proximal rat small intestine throughout the luminal concentration range studied and complete intestinal absorption was expected. There was an increase of P_{eff} from $0{\cdot}42 \times 10^{-4}$ cm s^{-1} to $0{\cdot}80 \times 10^{-4}$ cm s^{-1} ($P < 0{\cdot}05$) at concentrations from 4 to 400 mg L^{-1}, respectively. The observed concentration-dependent jejunal P_{eff} and fraction absorbed ($P < 0{\cdot}05$) of *R/S*-verapamil is consistent with the saturation of an efflux mechanism. When chlorpromazine (a P-glycoprotein inhibitor/substrate) was added the jejunal P_{eff} increased to $1{\cdot}47 \times 10^{-4}$ cm s^{-1}. There was no difference between the P_{eff} of the two enantiomers in any of these experiments. The efflux of *R/S*-norverapamil into the rat jejunum was high after intravenous administration of *R/S*-verapamil, suggesting extensive metabolism in the enterocyte.

In conclusion, both *R/S*-verapamil enantiomers are P-glycoprotein substrates, but there is no stereoselective transport of *R/S*-verapamil in the rat jejunum. The results also suggests that *R/S*-norverapamil is formed inside the enterocytes.

The role of P-glycoproteins in intestinal secretion, limiting oral drug absorption and increasing first-pass metabolism by CYP 3A4 in the gut wall, is of utmost pharmacological importance. The physiological function of P-glycoproteins has become evident by use of mice in-vivo with homozygously disrupted *mdr1a* gene (*mdr1a*$^{-/-}$). Those studies have demonstrated increased transport across the blood–brain barrier, lower systemic clearance, reduced faecal excretion and increased oral bioavailability (Schinkel et al 1994, 1997; Sparreboom et al 1997). Accordingly these reports suggest that P-glycoproteins serve to protect the organism from xenobiotics (Borst et al 1993).

The existence of a specific transmucosal secretion process for monoquaternary ammonium compounds, opposing drug diffusion in the absorptive direction, was proposed by Turnheim & Lauterbach (1977, 1980), who also presented a new concept in which intestinal secretion interacted with absorption; this might explain certain concentration-dependent absorption kinetics from guinea-pig intestine (Turnheim & Lauterbach 1980). For other β-adrenoceptor antagonists, celiprolol, talinolol, acebutolol and pafenolol, it has been suggested that the in-vivo dose-dependent bioavailability both in man and rats is a result of non-linear intestinal

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uptake (Lennernäs & Regardh 1993a; Gramatte et al 1996; Wetterich et al 1996). In bile-cannulated rats it was reported that intestinal secretion accounts for 25% of the total elimination of pafenolol (Lennernäs & Regardh 1993b). In the Caco-2 model it was shown that the transport of celiprolol, a P-glycoprotein substrate, was inhibited by acebutolol, propranolol, pafenolol and atenolol (Karlsson et al 1993). Active intestinal secretion of numerous drugs has also been reported in the rat small intestine (Terao et al 1996).

It has been reported that most substrates for P-glycoproteins are also substrates for CYP3A4 (De Waziers et al 1990; Wacher et al 1995). This co-operation might cause local recycling of a substrate in the enterocyte and, therefore, increase first-pass metabolism in the gut as a consequence of the reduced functional absorption rate across the intestinal epithelium. Recently reported data suggested that the P-glycoprotein content of the duodenum and CYP 3A4 activity in the liver (erythromycin breath test) explained most of the inter-individual variation of oral pharmacokinetics of cyclosporine (CL/F and C_{max}) seen in 25 kidney transplant patients (Lown et al 1997).

Verapamil is a lipophilic drug (log D 2·7, octanol–water; pH 7·4; MW 455 Da) which has been extensively used as a model substrate for P-glycoproteins in cancer cells (Stein 1997; Winiwarter et al 1997). Verapamil has been reported to have a rather high affinity for P-glycoproteins (Stein 1997). With regard to intestinal transport, use of excised intestinal segments in the Ussing chamber (in-vitro) has led to reports that the racemate is a substrate for some efflux mechanisms (Saitoh & Aungst 1995). However, so far no in-situ animal studies have investigated the transport and metabolism of the two enantiomers of both verapamil and norverapamil in the intestinal tissue when the drug is administered to both sides of the intestinal barrier.

The main purpose of this study was to investigate the possibility of stereoselective P-glycoprotein-mediated transport of *R*/*S*-verapamil in the rat jejunum.

Materials and Methods

Chemicals

Methanol and 2-propanol were obtained from Merck (Darmstadt, Germany). *R*/*S*-Verapamil and *R*/*S*-norverapamil were gifts from Knoll AG, (Darmstadt, Germany). All other chemicals were of analytical grade.

Animals

Male Charles River rats (Crl:CD(SD)BR), 250–300 g, were purchased from Charles River Sverige AB (Uppsala, Sweden). They were housed at the Biomedical Centre, University of Uppsala for one week before the experiment with a 12-h light–dark cycle and free access to food and water. After overnight fast with water freely available the rats were anaesthetized by intraperitoneal injection of Inactin (thiobutabarbital sodium; 120 mg kg^{-1}). Temperature was maintained at 37°C using a CMA-150 animal warmer. To facilitate breathing a tube was introduced into the trachea (PE-205).

Analytical methods

Enantioselective HPLC of *R*/*S*-verapamil and *R*/*S*-norverapamil was performed on a Chiral AGP column (4 mm × 150 mm; Chrometech, Stockholm, Sweden) with a Chiral AGP pre-column (3 mm × 10 mm). The pump was a Shimadzu (Kyoto, Japan) LC-9A. The mobile phase was pH 7·6 phosphate buffer with an ionic strength of 0·01 containing 22% (v/v) acetonitrile at 30°C and the flow rate was 1·0 mL min^{-1}. Temperatures above room temperature (22°C) were achieved by heating the column and the mobile phase in a heating bath (type JB1; Grant Instruments, Cambridge, UK). Compounds were detected with a Jasco (Tokyo, Japan) FP-920 fluorescence detector with an excitation wavelength of 232 nm and an emission wavelength of 310 nm. The perfusate samples were diluted with mobile phase and 50·1 μL were injected on to the column.

Plasma (100 μL) was mixed with diethyl ether (3 mL) and sodium hydroxide (4 M; 100 μL), vortex mixed for 2 min and then centrifuged at 2500 rev min^{-1} for 10 min on a Hettich (Tuttlingen, Germany) EBA 30. The ether phase was removed and an additional extraction was performed with diethyl ether (3 mL). The ether fractions were combined, evaporated under a stream of nitrogen until dryness, dissolved in mobile phase (200 μL), and 50·1 μL was injected on to the column. The limits of quantification (LOQ) (±s.d.) for *R*-verapamil were $5·5 \pm 0·3$ ng mL^{-1} in perfusate and 55 ± 3 ng mL^{-1} in plasma; for *S*-verapamil they were $5·5 \pm 0·4$ ng mL^{-1} in perfusate and 55 ± 5 ng mL^{-1} in plasma. The LOQ (±s.d.) for *R*-norverapamil were $2·9 \pm 0·2$ ng mL^{-1} in perfusate and $58·8 \pm 6$ ng mL^{-1} in plasma; for *S*-norverapamil they were $2·9 \pm 0·1$ ng mL^{-1} in perfusate and $58·8 \pm 5$ ng mL^{-1} in plasma.

The concentrations of [^{3}H]D-glucose and [^{14}C]PEG 4000 in the intestinal perfusate were determined by liquid-scintillation counting for

10 min (Mark III, Searle Analytic, Des Plaines, IL), as described elsewhere (Fagerholm et al 1996).

Intestinal perfusion solution
The intestinal perfusion solution consisted of potassium chloride 5·4 mM, sodium chloride 30 mM, mannitol 35 mM, D-glucose 10 mM and PEG 4000 1 g L^{-1}, all dissolved in phosphate buffer (70 mM, pH 6·5). Polyethylene glycol labelled with ^{14}C ([^{14}C]PEG 4000) and tritiated glucose ([^{3}H]D-glucose), from Amersham Laboratories (Buckinghamshire, UK) were added to the perfusion solution as, respectively, a volume marker with an activity of 2·5 μCi L^{-1} and absorption marker with an activity of 10 μCi L^{-1}.

Study groups
The study was performed on six separate groups of four rats. Rats in groups 1–4 were only perfused in the jejunum. Rats in groups 5 and 6 were given *R/S*-verapamil intravenously and at the same time the jejunum was perfused.

The concentrations of *R/S*-verapamil in perfusate entering the jejunal segment in groups 1–3 were 4, 40 and 400 mg L^{-1}, respectively. Group 4 received *R/S*-verapamil at an inlet perfusate concentration of 400 mg L^{-1} together with 10 mM chlorpromazine. Chlorpromazine has previously been used as a P-glycoprotein substrate/inhibitor for in-vitro intestinal transport studies (Saitoh & Aungst 1995).

Groups 5 and 6 received *R/S*-verapamil intravenously as a short bolus infusion of 300 μg min^{-1} kg^{-1} for 5 min, followed by a constant intravenous infusion of 20·2 μg min^{-1} kg^{-1} for 100 min. In those rats the jejunal segments were simultaneously single-pass perfused with drug-free perfusion solution in group 5 and with 10 mM chlorpromazine added to the perfusion solution in group 6. Arterial blood samples (400 μL) were collected 30, 52·5, 67·5, 82·5 and 97·5 min after the start of the intravenous infusion of *R/S*-verapamil in groups 5 and 6. The blood samples were immediately centrifuged at 10 000 rev min^{-1} for 10 min, and the plasma fractions were frozen at −18°C before analysis.

Jejunal perfusion and intravenous administration
The abdominal cavity was opened by midline incision and a 10-cm segment of the jejunum was catheterized with plastic tubing. Care was taken not to disturb the blood flow of the isolated segment. The inlet tubing had a loop inside the intestinal cavity of the rat to preheat the perfusion solution before it entered the jejunal segment. Initially the segment was rinsed with isotonic saline (37°C) until the perfusate leaving the segment was clear. Perfusion was started by giving a bolus of 4 mL of the perfusion solution, followed by 105 min perfusion with a sampling interval of 15 min during steady state. The flow rate was 0·2 mL min^{-1} and was maintained with a calibrated syringe pump (Harward Apparatus 22, South Natick, USA). All syringes and sampling vials were weighed before and after use in the perfusion experiments. The sampled fractions were quantitatively collected on ice and immediately frozen (−18°C) before analysis. After perfusion the jejunal segment was rinsed with 12–15 mL isotonic saline to collect the perfusion solution still in the segment for mass–balance calculations. The animals were killed by intraperitoneal injection of pentobarbital sodium.

These animal experiments were approved by the ethics committee for the use of experimental animals at Tierps tingsrätt (Tierp, Sweden) with approval number C246/95. Those rats that received verapamil intravenously were catheterized with plastic catheters (PE-50) in the jugular vein and carotid artery for drug administration and sample collection, respectively.

Data analysis
Calculations of the transport variables were made from the outlet concentrations of the perfusate leaving the jejunal segment during steady state. Steady-state conditions were considered to have been reached when the concentration of [^{14}C]PEG 4000 leaving the segment was stable. Steady state in the outlet perfusate was reached within 45 min in all rats. The net water flux (NWF) cm^{-1} in the jejunal segment was calculated using equation 1:

$$NWF = (1 - PEG_{out}/PEG_{in})Q_{in}/L \qquad (1)$$

where PEG_{in} and PEG_{out} are respectively the inlet and outlet concentrations of [^{14}C]PEG 4000. Q_{in} is the flow rate entering the segment (0·2 mL min^{-1}) and L is the length of the intestinal segment (10 cm).

The effective jejunal permeability (P_{eff}) was calculated according to the parallel-tube model which has been used in other animal studies (Levitt et al 1988; Fagerholm et al 1996):

$$P_{eff} = -Q_{in} \times \ln(C_{out}/C_{in})/(2\pi rL) \qquad (2)$$

where C_{in} and C_{out} are the inlet and outlet concentrations corrected for net water flux and $2\pi rL$ is the cylinder surface area of the jejunal segment of length L (10 cm) and intestinal radius r (0·18 cm).

The fraction absorbed (f_a) was calculated by use of equation 3, assuming that the fraction of the drug that disappeared during passage through the segment has been absorbed (Lennernäs et al 1992).

$$f_a = 1 - (C_{out} \times PEG_{in})/(C_{in} \times PEG_{out}) \quad (3)$$

Statistical analysis for groups 1–4 was performed using a one-factor analysis of variance test, and the difference between groups was analysed with the Fisher PLSD test and the Scheffe *F*-test. Statistical analysis of the difference between results from groups 5 and 6 was performed using an unpaired two-tailed Student's *t*-test. Data are presented as mean ± s.d.

Results and Discussion

In this study we used a validated intestinal perfusion model in rats which has been reported to correlate well and predict intestinal permeability and extent of oral drug absorption in man (Fagerholm et al 1996). P_{eff} was determined from the luminal disappearance rate of each enantiomer of verapamil (equation 2 and Table 1). Intestinal secretion was investigated by giving the verapamil racemate intravenously and simultaneously monitoring the secretion of the enantiomers of drug and of one metabolite in the perfused jejunal segment. The average effective jejunal permeability (P_{eff}) and fraction absorbed (f_a) for both *R*- and *S*-verapamil increased significantly when the inlet perfusate concentration was increased from 4 and 400 mg L^{-1} (Tables 1 and 2). This non-linear increase in P_{eff} and f_a is probably because of saturation of P-glycoprotein-mediated efflux. There was a slight difference between results for the enantiomers at the lowest concentration (4 mg L^{-1}), but at 40 and 400 mg L^{-1} results were similar (Tables 1 and 2). Additional evidence that both *R*- and *S*-verapamil are P-glycoprotein substrates in the rat intestine is the increased P_{eff} and f_a obtained after co-administration of chlorpromazine (a P-glycoprotein inhibitor/substrate) (Tables 1 and 2) (Saitoh & Aungst 1995). All these data show that the specificity of P-glycoprotein in the apical membrane of the enterocyte cannot discriminate between the enantiomers of verapamil. This observation further supports the general view that the protein has very broad substrate specificity (Stein 1997). The absorption variables for D-glucose were similar throughout the study, which demonstrates that the active absorption of D-glucose was unaffected by increased luminal concentrations of *R*/*S*-verapamil and by addition of chlorpromazine (Table 2). Groups 5 and 6 received *R*/*S*-verapamil as an intravenous bolus dose followed by a constant infusion (Figure 1A). The increase in plasma concentration of *R*/*S*-verapamil in group 6 is probably a result of inhibition of the metabolism of *R*/*S*-verapamil by chlorpromazine because both drugs are transformed by the same enzyme (CYP3A4) in man (Cashman et al 1993; Fromm et al 1996) (Figure 1B). It is unclear if this

Table 1. The effective jejunal permeability (P_{eff}) of *R*/*S*-verapamil at different concentrations and in combination with chlorpromazine studied with single-pass perfusion.

Inlet concentration of *R*/*S*-Verapamil (mg L^{-1})	P_{eff} (10^{-4} cm s^{-1})	
	R-Verapamil	*S*-Verapamil
4	0·55 ± 0·13	0·42 ± 0·13
40	0·56 ± 0·15	0·54 ± 0·17
400	0·8 ± 0·17	0·81 ± 0·21
400 + chlorpromazine (10 mM)	1·47 ± 0·36	14·6 ± 0·35

Values are means ± s.d. There was no significant difference between results obtained for the enantiomers within groups. One-factor analysis of variance gave a total statistical difference with $P < 0·003$ for all the groups. Between the groups statistical analysis showed a difference ($P < 0·05$) between groups 1 and 3, and between group 4 and all the other groups.

Table 2. Absorption variables for *R*/*S*-verapamil, D-glucose and water in rat jejunal perfusions.

Inlet concentration of *R*/S-verapamil (mg L^{-1})	*R*-Verapamil Fraction absorbed (%)	*S*-Verapamil Fraction absorbed (%)	D-Glucose P_{eff} (10^{-4} cm s^{-1})	D-Glucose Fraction absorbed (%)	Net water flux (mL h^{-1} cm^{-1})
4	17·7 ± 3·9*	13·8 ± 3·9‡	0·57 ± 0·21	18·2 ± 6·4	0·03 ± 0·05
40	18·7 ± 2·9†	18·1 ± 4·1§	0·50 ± 0·24	17·4 ± 8·3	0·07 ± 0·07
400	28·1 ± 11·3*†	27·4 ± 12·4‡§	0·46 ± 0·24	17·4 ± 8·6	0·04 ± 0·06
400 + chlorpromazine 10 mM	42·7 ± 6·7*†	42·7 ± 6·1‡§	0·60 ± 0·19	20·7 ± 6·3	0·02 ± 0·07

Values are means ± s.d. *†‡§ $P < 0·5$ statistically different from groups with the same symbols.

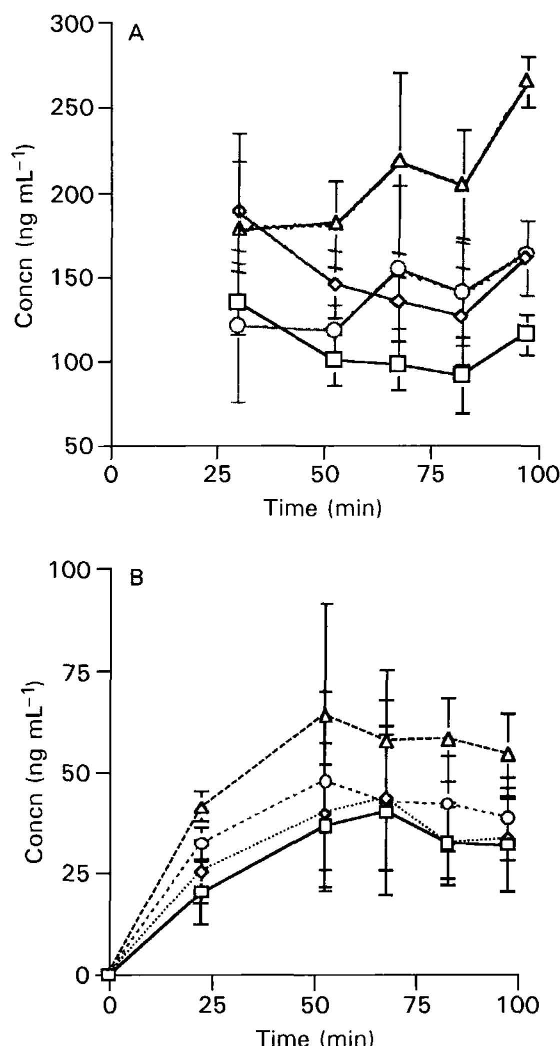

Figure 1. Plasma (A) and outlet perfusate (B) concentrations of *R*/*S*-verapamil after intravenous infusion of *R*/*S*-verapamil and simultaneous single-pass perfusion of the jejunum with and without chlorpromazine. In group 5 the rats (n = 4) were perfused with a control perfusion solution (without chlorpromazine), and in group 6 (n = 4) chlorpromazine was added to the perfusion solution. □ *R*-Verapamil (group 5); ◇ *S*-verapamil (group 5); ○ *R*-verapamil (group 6); △ *S*-verapamil (group 6).

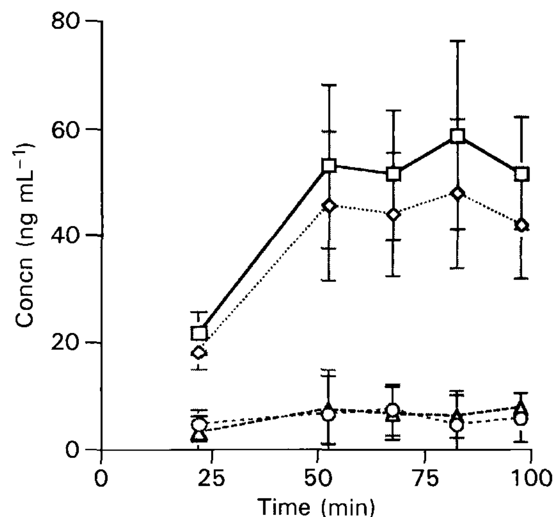

Figure 2. Perfusate concentrations of *R*/*S*-norverapamil after intravenous infusion of *R*/*S*-verapamil and simultaneous single-pass perfusion of the jejunum with and without chlorpromazine. In group 5 the rats (n = 4) were perfused with a control perfusion solution (without chlorpromazine), and in group 6 (n = 4) chlorpromazine was added to the perfusion solution. Levels of *R*/*S*-norverapamil in the outlet perfusate from groups 5 and 6 were statistically different ($P < 0.01$). □ *R*-Norverapamil (group 5); ◇ *S*-norverapamil (group 5); ○ *R*-norverapamil (group 6); △ *S*-norverapamil (group 6).

is true for the rat, but the reduced secretion of *R*/*S*-norverapamil in rats receiving chlorpromazine indicates that the same enzyme(s) is responsible for the transformation of both drugs in the rat (Figure 2). When the perfusate concentrations of *R*/*S*-verapamil were related to the unbound plasma concentrations they were approximately three to five times higher (Table 3). This suggests that the drug is transported against a concentration gradient by a carrier-mediated process. The unbound plasma concentrations were calculated from values of the free fractions, 0·1 and 0·06 for *R*- and *S*-verapamil, respectively, and they were adopted from a previously reported in-vitro plasma protein-binding study (Robinson & Mehvar 1996). However, when chlorpromazine was present in the perfusion solu-

Table 3. Concentrations of *R*/S-verapamil in plasma and perfusate for the groups 5 and 6.

	Group 5		Group 6	
	R-Verapamil	*S*-Verapamil	*R*-Verapamil	*S*-Verapamil
Concentration in plasma (ng mL^{-1})	103 ± 18·9	143 ± 25·9	146 ± 35·0	219 ± 46·1
Concentration in perfusate (ng mL^{-1})	36·0 ± 15·4	37·8 ± 17·7	43·3 ± 16·1	59·2 ± 17·9
Concentration in perfusate/ concentration in plasma	0·37 ± 0·17	0·28 ± 0·14	0·32 ± 0·15	0·28 ± 0·11
Unbound concentration in perfusate/ unbound concentration in plasma	3·55 ± 1·66	4·51 ± 2·32	3·0 ± 1·46	4·6 ± 1·79

Values are means ± s.d. The unbound concentration was calculated using free fractions of 0·10 and 0·06 for *R*- and *S*-verapamil, respectively (Robinson & Mehvar 1996). Values in parentheses are the standard deviations. Group 5 was given intravenous infusion of verapamil and blank perfusion solution; group 6 was given intravenous infusion of verapamil and perfusion solution containing 10 mM chlorpromazine.

tion the ratio of the perfusate concentration to the calculated free concentration of *R*/*S*-verapamil in plasma were similar to the value obtained for rats that only received the blank perfusion solution (Table 3). These data show that chlorpromazine given as a single-pass perfusion in a 10-cm jejunal segment did not affect the overall exsorption but reduced systemic clearance for both enantiomers. One plausible explanation of the lack of an observed effect in the outlet perfusate might be the occurrence of a competitive plasma-protein binding interaction, because both drugs are bound to albumin and α-acid glycoproteins (McGowan et al 1983; Verbeek et al 1983; Mehvar & Reynolds 1996). This could increase the passive efflux pathway of *R*/*S*-verapamil, and therefore mask the reduced active efflux by P-glycoproteins. P-Glycoprotein-mediated secretion transport (basolateral to apical) of verapamil (racemic) and chlorpromazine has been demonstrated in-vitro using diffusion cells with rat intestinal segments (Saitoh & Aungst 1995). Our new results confirm earlier in-vitro data that P-glycoproteins do not discriminate between the enantiomers of verapamil (Plumb et al 1990).

The P_{eff} in-situ in rats has been shown to correlate well with the extent of intestinal absorption of drugs in-vivo in man (Fagerholm et al 1996). Even if the P_{eff} of *R*/*S*-verapamil in this study was concentration-dependent, both enantiomers are highly permeable drugs and will be completely absorbed at all luminal concentrations. Instead, our data support the hypothesis that the concentration-dependent permeability reflects changes in local absorption rate which will determine the presentation rate of the drug for the intracellular gut enzymes (Lown et al 1997). The only evidence for the relevance of P-glycoproteins in intestinal absorption and bioavailability is shown for paclitaxel in mice with homozygously disrupted *mdr1a* gene (Sparreboom et al 1997). The increased bioavailability of that P-glycoprotein substrate was explained as being a result of the lack of P-glycoproteins in the apical enterocyte membranes. However, these knock-out animals might also be affected by other physiological factors, even if the same group consider them to have a normal physiology (Schinkel et al 1994, 1997). One plausible reason why the extent of intestinal absorption is affected for paclitaxel and not verapamil might be a combination of lower membrane concentration and slower membrane diffusion of paclitaxel (MW 853·9 Da) compared with *R*/*S*-verapamil. These properties might result in less saturation of P-glycoproteins, because the binding sites are suggested as being located within the membrane phase of the cell (Higgins & Gottesman 1992).

The levels of *R*/*S*-norverapamil in plasma were below the limit of detection in all plasma samples after intravenous administration. However, the concentrations of *R*/*S*-norverapamil in the perfusate samples were approximately the same as for the parent drug in the control rats, and about ten times lower for the rats receiving chlorpromazine (Figure 2). The observation that *R*/*S*-norverapamil could not be detected in plasma, but was readily assayed in the perfusate, suggests that the compound is metabolized in the enterocyte even after an intravenous dose. The appearance of the metabolites in the intestinal lumen could be mediated by carrier-mediated secretion (by P-glycoproteins or other efflux proteins) as well as by passive exsorption.

In summary, the jejunal P_{eff} values for both enantiomers of *R*/*S*-verapamil were concentration-dependent and increased further in the presence of chlorpromazine in the intestinal lumen. Even though the P_{eff} of *R*/*S*-verapamil was concentration-dependent both enantiomers are regarded as high permeable drugs ($>0{\cdot}5 \times 10^{-4}$ cm s^{-1}), which implies that intestinal absorption will be complete (Fagerholm et al 1996). The overall results in this study support the view that *R*- and *S*-verapamil are indeed P-glycoprotein substrates in the gut enterocyte, and that there is no difference between the two enantiomers in the P-glycoprotein mediated transport. The low bioavailability of *R*/*S*-verapamil is instead a result of extensive first-pass metabolism in the liver and gut wall (Fromm et al 1996). However, P-glycoproteins might be a factor affecting intestinal drug absorption for other drugs with different physicochemical properties, and therefore having lower membrane concentrations and slower diffusion across the membrane phase.

This biotransformation of *R*/*S*-verapamil in the gut wall is to some extent performed by CYP3A-equivalent enzymes expressed in rat enterocytes (Kroemer et al 1992; Thummel et al 1996). However, the exsorption of *R*/*S*-norverapamil into the jejunal lumen after intravenous administration of *R*/*S*-verapamil suggests significant metabolic capacity located in the rat intestinal tissue. The reduced exsorption of *R*/*S*-norverapamil when chlorpromazine is co-perfused in the jejunal segment is probably because of inhibited metabolism in the enterocyte, but it might also be because of inhibited active efflux because it has been reported that *R*- and *S*-norverapamil are substrates for P-glycoproteins.

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