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Regional Intestinal Permeability in Rats of Compounds with Different Physicochemical Properties and Transport Mechanisms

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Abstract

Because the absorption of orally administered drugs depends on intestinal permeability, we have investigated how absorptive capacity varies from the proximal to distal intestine in rats. The effective permeabilities of compounds with a range of physicochemical properties and different absorption mechanisms were estimated by use of a previously validated in-situ, single-pass perfusion model.

The low colonic permeabilities of D-glucose and L-dopa indicate the absence or low capacity of the glucose- and amino-acid-transporters in this region. With the exception of the small and moderately lipophilic non-steroidal anti-inflammatory drug, naproxen, for which permeability was maintained throughout the intestine, the passive intestinal permeabilities for hydrophilic and lipophilic drugs were approximately twice as high in the jejunum and ileum as in the colon. These observations are in accord with those made in recent studies. However, the reasons for the high colonic permeability of non-steroidal anti-inflammatory drugs, and results obtained in previous animal experiments demonstrating that the colon is the region of the intestine with the highest absorptive capacity were not fully clarified.

These data show that the permeability to hydrophilic and lipophilic drugs decreases along the intestine, whereas it is maintained throughout the intestine for the small and moderately lipophilic naproxen. Further investigations are required to clarify the interplay between membrane composition, fluidity and permeability under various conditions in different absorption models.

To optimize absorption of orally administered drugs it is important to determine the permeability characteristics of drug candidates in various intestinal regions of different absorption models. In consistence with the decline in the absorptive area, mesenteric blood flow, paracellular pore size and carrier-mediated transport capacity along the intestine, the intestinal permeability for hydrophilic compounds has been observed to decrease, both in man in-vivo and in animals in-situ and in-vitro (Chadwick et al 1977; Schultz & Winne 1987; Artursson et al 1993; Sinko & Hu 1996). The colon has been shown to have a high absorptive capacity for highly lipid-soluble compounds. This was exemplified in a recent study on man by Abrahamsson et al (1996), who found that the in-vivo uptake of metoprolol, a β-blocker of intermediate lipophilicity (MW 267; log D 0·0 for octanol-water at pH 7·4), was considerable from both the small and large intestine (Sandberg 1994; Abrahamsson et al 1996). Some animal in-situ and in-vitro studies have demonstrated that the colon is the most permeable region of the gastrointestinal tract for such drugs (Jezyk et al 1992; Narawane et al 1992; Ungell et al 1996). It was suggested that this might be because the lipid composition of the colonocyte membrane favours the uptake of lipid-soluble substances (Ungell et al 1996). This has not, however, been fully clarified because some authors have observed reduced or uniform permeability for lipophilic compounds along the rat intestine (Kimura et al 1994), whereas others have found that the colon is the region of the animal intestine with the highest permeability for hydrophilic solutes (Hollander et al 1989; Krugliak et al 1994; Ma et al 1995). In man, for example, it has been shown that antipyrine (MW 188; log D 0·4 in octanol-water at pH 7·4) has a 5–6 times higher effective permeability (P_{eff}) in the jejunum than in the colo-rectum (Lennernäs et al 1995). Further investigations and validations of the commonly used absorption models are, therefore, required if variations in permeability for different solutes along the intestine is to be better characterized.

This study has focused on investigation of regional P_{eff} for seven compounds with different physicochemical properties and transport mechanisms in the rat. We used an established in-situ single-pass perfusion approach; it has previously been shown that estimates of jejunal P_{eff} for passively transported compounds obtained by this approach correlate strongly with those obtained in-vivo in the jejunum in man (Fagerholm et al 1996a).

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Materials and Methods

Perfusion experiments and study design

Intestinal segments of rat jejunum (n = 6), ileum (n = 6) and colon (n = 6) were single-pass perfused with an isotonic solution (pH 6·5) at a flow rate of 0·2 mL min^{-1}. The compounds investigated had different physicochemical properties and absorption mechanisms (Table 1). The rats were anaesthetized by intraperitoneal injection of inactin-byk (thiobutabarbital sodium, 120 mg kg^{-1}), an anaesthetic previously shown to have little or no influence on intestinal permeability for passively and actively absorbed compounds (Yuasa et al 1993; Fagerholm et al 1996a). A detailed description of the experimental procedure and the composition of the perfusion solution is presented elsewhere (Fagerholm et al 1996a). The duration of the jejunal perfusions was 90 min, and the perfusion solution during these experiments contained [^{3}H]D-glu-

Table 1. Physicochemical properties and inlet concentrations of eight different compounds.

Drug	Molecular weight	pK_a	Lipophilicity*	Inlet concn (mM)
Passive absorption				
Antipyrine	188	1·5† (base)	0·4†	1·05
Atenolol	266	9·6† (base)	−1·8†	0·83
Fluvastatin	411	4·6‡ (acid)	1·9‡	0·16
Metoprolol	267	9·7† (base)	0·0†	0·58
Naproxen	230	4·4† (acid)	0·1†	1·8
PEG 4000	4000	– (–)	n.i.	0·25
Carrier-mediated absorption				
D-Glucose	180	– (–)	−3·0†	10
L-Dopa¶	197	2·3, 8·7, 9·7, 13·4† (acid)	n.i.	2·5

Compound behaviour was studied by in-situ single-pass perfusion in the rat intestine. The pH and osmolality of the solutions entering the test segments were 6·5 and 290 mOsm L^{-1}, respectively, except where indicated. *log D Octanol-water, pH 7·4. †From Fagerholm et al 1996a. ‡From Lindahl et al 1996. log D for fluvastatin at pH 6·5. ¶Jejunal P_{eff} for L-dopa was obtained at pH 7·4. n.i. = no information available.

cose, fluvastatin and [^{14}C]PEG 4000. Estimates of jejunal P_{eff} for antipyrine, atenolol, metoprolol, naproxen and L-dopa have previously been obtained in the rat (Fagerholm et al 1996a). Ileal and colonic perfusions lasted 180 min, divided into two randomized periods (A and B) of 90 min each. The perfusion solutions, always protected from light, contained: (period A) antipyrine, [^{3}H]D-glucose, L-dopa, naproxen and [^{14}C]PEG 4000; and (period B) atenolol, [^{3}H]D-glucose, fluvastatin, metoprolol and [^{14}C]PEG 4000. To prevent degradation of L-dopa, the decarboxylase inhibitor benserazide was added to the solution in period A. All samples of perfusion solution and intestinal perfusate were stored at −70°C until analysis.

Intestinal barrier function (viability) was assessed by determining the steady-state recovery of [^{14}C]PEG 4000, and by inspection of the steady-state P_{eff} of [^{3}H]D-glucose (marker for active absorption) and antipyrine (marker for passive absorption and indicator for extensive changes in mesenteric blood flow) (Schultz & Winne 1987; Fagerholm et al 1996a).

There was no adsorption of the investigated compounds by the inlet and outlet plastic tubes, nor were they degraded to any significant extent during the experiments (Fagerholm et al 1996a). The concentrations of antipyrine, atenolol, fluvastatin, L-dopa, metoprolol and naproxen in perfusion solutions and perfusate were determined using previously validated HPLC methods (Fagerholm et al 1996a; Lindahl et al 1996). Concentrations of [^{3}H]D-glucose and [^{14}C]PEG 4000 were assessed by liquid-scintillation counting. Approval for these studies was given by the Uppsala Animal Research Ethics Committee (application number C246/95).

Data analysis

The effective intestinal permeability (P_{eff}, cm s^{-1}) and intestinal net water flux (J_{water}, mL h^{-1} cm^{-1}) at equilibrium were determined according to equations 1 and 2, respectively (Komiya et al 1980; Fagerholm et al 1996a):

$$P_{eff} = [-Q_{in} \cdot \ln(C_{out}/C_{in})]/A \quad (1)$$

$$J_{water} = [1 - ([PEG]_{out}/[PEG]_{in})] \cdot Q_{in}/L \quad (2)$$

where C_{in} and C_{out} are the inlet and fluid-transport-corrected outlet solute concentrations, respectively, and $[PEG]_{in}$ and $[PEG]_{out}$ are, respectively, the inlet and outlet concentrations of the water flux marker [^{14}C]PEG 4000. Q_{in} is the perfusion flow rate, and A is the mass-transfer surface-area within the intestinal segment—assumed to be the surface area of a cylinder of length (L) 10, 10–12 and 2–4 cm, and radius (r) 0·18, 0·18 and 0·25 cm in the jejunum, ileum and colon, respectively (Komiya et al 1980; Kararli 1995).

Analysis of variance and Student's paired *t*-test, respectively, were used to investigate statistical differences between intestinal regions and perfusion periods (periods A and B in the ileal and colonic experiments). Throughout this paper the P_{eff} and J_{water} values are presented as mean ± standard deviation (s.d.).

Results and Discussion

Complete recovery of PEG 4000, and stable water fluxes and P_{eff} coefficients, with time, for compounds transported both passively and by a carrier-mediated mechanism indicated that intestinal barrier function (viability) was maintained in all experiments. Ileal P_{eff} estimates for passively transported drugs were generally slightly lower, but not statistically different, from those obtained in the jejunum, indicating that passive permeability for compounds with molecular weights between 200 and 400 was reasonably uniform along the rat small intestine in-situ (Table 2, Fig. 1). This is in good agreement with previous studies in animals and man, and in line with small differences between the absorptive area and blood flow of the proximal and distal small intestine (Chadwick et al 1977; Schultz & Winne 1987; Kimura et al 1994; Sinko & Hu 1996). The P_{eff} of D-glucose was also similar throughout the rat small intestine, whereas for L-dopa, which utilizes the carrier-mediated transport system for large neutral amino acids, the P_{eff} in the ileum was approximately 40% lower than that in the jejunum ($P < 0·01$) (Table 2, Fig. 1) (Lennernäs et al 1993). The lower capacity of the amino-acid carrier to transport L-dopa in the distal region of the rat small intestine might be because of lower expression of this transport protein. It is, however, not likely that the estimated P_{eff} values were affected by non-linear kinetics, because the perfusate concentrations of L-dopa (2·5 mM) and of D-glucose (10 mM) were below the reported K_m values of each carrier system (5–10 and 10–20

Table 2. Mean ± s.d. effective intestinal permeability for seven different compounds and intestinal net water flux in the single-pass perfused rat intestine in-situ. A negative intestinal net water flux indicates net water absorption.

Drug	Effective permeability ($\times 10^{-4}$ cm s^{-1})			Statistics		
	Jejunum	Ileum	Colon	Jejunum compared with ileum	Jejunum compared with colon	Ileum compared with colon
Passive absorption						
Antipyrine	1·6 ± 0·40*	1·3 ± 0·23	0·75 ± 0·66	n.s.	$P < 0.001$	$P < 0.05$
Atenolol	0·06 ± 0·07*	0·01 ± 0·01	0·02 ± 0·06	n.s.	n.s.	n.s.
Fluvastatin	1·6 ± 0·44	1·3 ± 0·22	0·99 ± 0·58	n.s.	$P < 0.05$	n.s.
Metoprolol	0·33 ± 0·20*	0·53 ± 0·22	0·09 ± 0·15	n.s.	$P < 0.05$	$P < 0.01$
Naproxen	2·1 ± 0·41*	2·1 ± 0·40	2·5 ± 1·3	n.s.	n.s.	n.s.
Carrier-mediated absorption						
D-glucose	1·4 ± 0·38	1·4 ± 0·27	0·08 ± 0·11	n.s.	$P < 0.001$	$P < 0.001$
L-dopa	2·0 ± 0·63*	1·2 ± 0·27	0·02 ± 0·04	$P < 0.01$	$P < 0.001$	$P < 0.001$
Net water flux, J_{water} (mL h^{-1} cm^{-1})	−0·056 ± 0·059†	−0·066 ± 0·046	−0·037 ± 0·155	n.s.	n.s.	n.s.

*From Fagerholm et al (1996a). n.s. = not significant. †Includes estimates obtained in Fagerholm et al (1996a).

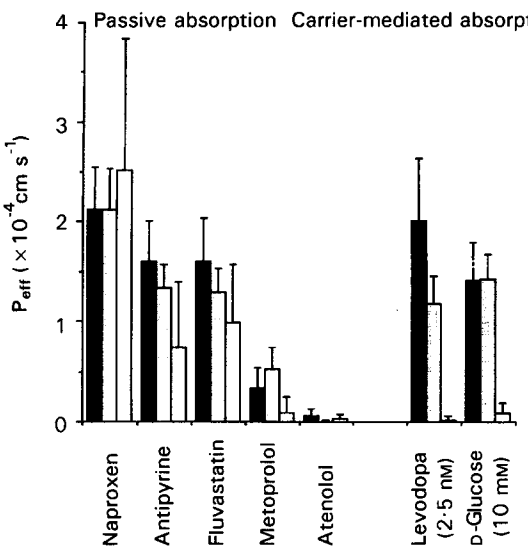

FIG. 1. Mean ± s.d. effective permeability (P_{eff}) of the rat jejunum (■), ileum (▒) and colon (□) for compounds with different physicochemical properties and transport mechanisms.

mM, respectively; Alpers 1987; Pappenheimer 1993). Nor is it believed that the decrease in the P_{eff} of L-dopa along the small intestine was a result of regional differences in paracellular uptake because it is suggested that this route does not contribute to quantitative intestinal absorption of this and other compounds with a MW > 200 (Lennernäs 1995; Schwartz et al 1995; Fagerholm et al 1996b).

The P_{eff} in the colon for the hydrophilic to moderately lipophilic passively absorbed compounds antipyrine, metoprolol and atenolol was 50–70% lower than in the jejunum, which is consistent with several other reports of results from animals and man, and with the lower absorptive area and blood flow in this region (Table 2 and Fig. 1) (Chadwick et al 1977; Schultz & Winne 1987; Artursson et al 1993; Kimura et al 1994; Lennernäs et al 1995; Sinko & Hu 1996). However, these observations are in conflict with data obtained by other groups, who observed a higher P_{eff} in the colon than in the small intestine for hydrophilic compounds (Hollander et al 1989; Krugliak et al 1994; Ma et al 1995). The reason(s) for their discrepant results is not clear, but as previous in-situ and in-vitro animal studies have shown, it might be explained by a loss of intestinal barrier function during the experimental procedure (Fagerholm et al 1996a). The approximately 20 and 100 times lower colonic P_{eff} values for D-glucose and L-dopa, respectively, compared with P_{eff} values for the jejunum are in agreement with in-vivo observations for D-glucose in the intestine in man, and is indicative of the absence of, or the low capacity of, the glucose- and amino-acid-transporters in the large intestine (Lennernäs et al 1995). It also confirms reported data showing low uptake of L-dopa after rectal administration (Eisler et al 1981). The decrease in P_{eff} from the proximal to distal rat intestine was less pronounced for the more lipophilic fluvastatin, whereas the P_{eff} for naproxen, a non-steroidal anti-inflammatory drug which also has lipophilic properties, was similar in the jejunum, ileum and colon (Table 2, Fig. 1). These observations are supported by Kimura et al (1994) who found that a small drug of intermediate lipophilicity (paracetamol, MW 151) was more rapidly absorbed from the small than the large intestine in rats, whereas the P_{eff} of a highly lipophilic compound (indomethacin, MW 358) was similar in these regions (Kimura et al 1994). The reasons for the different permeability patterns for hydrophilic and lipophilic compounds are not known, but might be because the composition of colonocyte membranes favours the uptake of lipophilic compounds, and because of the influence of the unstirred water layer adjacent to the intestinal wall which is a diffusional barrier shown to have a considerable influence on the P_{eff} for highly permeable compounds in the rat perfused intestine in-situ (Levitt et al 1984; Winne 1987). Other factors such as paracellular absorption and solvent drag are less probable, because in this rat model neither solvent drag nor high luminal levels of D-glucose (up to 80 mM) have been shown to influence the P_{eff} of compounds of this size (Fagerholm et al 1996b). Naproxen and indomethacin have previously been shown to cause damage to the intestinal mucosa, and thereby increase intestinal permeability (Somasundaram et al 1995). However, microscopic

examination showed that 1-h exposure to indomethacin did not damage the rat intestinal mucosa, and the jejunal P_{eff}-values of antipyrine, L-dopa, D-glucose and PEG 4000 obtained in the presence of 1·8 mM naproxen were no different from those obtained without naproxen (Fagerholm et al 1995; Somasundaram et al 1995). Other groups have previously observed in animals in-vitro and in-situ that the colon has a higher permeability for lipophilic compounds than does the small intestine (Jezyk et al 1992; Narawane et al 1992; Ungell et al 1996). The differences between these and our data might be explained by differences in cell-membrane composition—colonocyte membrane cholesterol content has been shown to vary widely between experimental set-ups (Brasitus & Dudeja 1985; Ungell et al 1996). This clearly demonstrates the further need for studies comparing membrane composition and fluidity with the transport properties of drugs with different physicochemical properties and transport mechanisms.

The data obtained in this study show that permeability to hydrophilic and lipophilic drugs decreases along the intestine, whereas it is maintained throughout the intestine for a small and moderately lipophilic non-steroidal anti-inflammatory drug, naproxen. The reasons for the high colonic permeability of naproxen and results obtained in previous animal experiments demonstrating that the colon is the region of the intestine with the highest absorptive capacity have not been fully clarified. The interplay between membrane composition, fluidity and permeability should be further investigated under various conditions in different absorption models.

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