International Journal of Pharmaceutics, 98 (1993) 83–92

IJP 03216

pH-dependent transepithelial transport of cephalexin in rabbit intestinal mucosa

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(Received 2 December 1992)
(Modified version received 20 February 1993)
(Accepted 22 February 1993)

Key words: Transepithelial transport; Cephalexin; Benzylpenicillin; pH dependence

Summary

Previous studies have demonstrated carrier-mediated cephalexin (CPX) transport in rat jejunum under basal conditions. The purpose of this study was to compare fluxes of cephalexin (CPX) and benzylpenicillin (BZP) across rabbit intestine to those reported in rat intestine, to determine whether these fluxes exhibit regioselectivity and to investigate the pH dependence of these fluxes. Fluxes of CPX were examined in rabbit jejunal, ileal and distal colonic mucosa in Ussing chambers. Concurrently, transepithelial (TE) potential difference, TE electrical conductance and mannitol fluxes were determined to evaluate tissue viability and integrity. Mucosal (m)-to-serosal(s) fluxes of CPX were ~ 3-times the s-to-m fluxes (0.66 vs 0.18% h^{-1} 3 cm^{-2}, respectively) in both jejunum and ileum under short-circuit conditions. Distal colonic m-to-s and s-to-m fluxes were ~ 0.18% h^{-1} 3 cm^{-2}. Inhibition of Na^+/K^+-ATPase activity with ouabain or incubation with the Na^+/H^+ exchange inhibitor, amiloride, reduced by 70 and 40%, respectively, the m-to-s fluxes of CPX in the ileum. Effects of changes in luminal pH on TE transport of CPX and mannitol were studied in ileal and colonic mucosa. At a luminal pH of 5.5, ileal m-to-s transport of CPX was increased to $2.85 \pm 0.12\%$ h^{-1} 3 cm^{-2} while mannitol flux and electrical properties were unchanged. In distal colon, pH 5.5 in the luminal bathing solution did not alter CPX or mannitol fluxes. pH dependent carrier-mediated transport was not exhibited by BZP. Thus CPX appears to be absorbed by a pH dependent, carrier-mediated mechanism confined to the small intestine whereas BZP absorption appears to occur by an entirely passive process.

Introduction

The intestinal peptide transporter is responsible for absorption of di- and tripeptides and numerous β-lactam antibiotics following oral administration (Humphrey, 1986; Smith et al., 1992). This peptide transporter has been shown to mediate the uptake of β-lactam antibiotics in vitro (Kimura et al., 1983; Nakashima et al., 1984; Hori et al., 1988). Because of its low specificity, this carrier can interact with a large number of structures. This feature of the carrier could be of great value in drug discovery by aiding in the design of peptidomimetic molecules with improved gastrointestinal absorption. However, this can only be achieved through a greater understanding of the mechanisms involved in the intestinal absorption of peptides. Previous studies have provided

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Abbreviations: m, mucosal; s, serosal; BBMV, brush border membrane vesicles; G_t, electrical conductance; I_{sc}, short-circuit current; PGE_1, prostaglandin E_1; KBR, bicarbonate-Ringer solution.

evidence for a model for transepithelial transport of di-and tripeptides. The existing evidence indicates that: (1) uptake at the apical membrane occurs by a proton-dependent carrier; (2) establishment and maintenance of the proton gradient across the apical cell membrane is due to a sodium/proton exchanger; (3) the driving force for sodium/proton exchange is the combined chemical gradients for sodium and protons and is dependent on maintenance of low intracellular Na^+ concentration by the basolaterally located Na^+/K^+-ATPase; and (4) exit of peptides across the basolateral membrane is due to an ill-defined mechanism which may also involve a proton-dependent carrier (Ganapathy and Leibach, 1985; Dyer et al., 1990). Subsequent studies have suggested the involvement of the apically located proton-dependent di-/tripeptide transporter in the transport and absorption of a number of related structures including angiotensin converting enzyme (ACE) inhibitors and renin inhibitors (Humphrey and Ringrose, 1986; Hu and Amidon, 1988; Kramer et al., 1990a,b,c).

The majority of studies which have examined uptake via the di-/tripeptide transporter have focused on disappearance of administered molecules from the lumen of perfused intestinal segments, transfer across everted intestinal sacs, or on uptake of molecules into brush border membrane vesicles (BBMV) (Kimura et al., 1983; Nakashima et al., 1984; Hori et al., 1988;). Frequently, rate of disappearance from luminal perfusate or uptake by BBMV is assumed to be equal to the rate of absorption and thus attempts are made to equate transport in this system to in vivo absorption (Hori et al., 1988). Although this approach may be adequate in some instances, biotransformation in the intestinal mucosa, binding and/or partitioning into the gut wall may result in overestimation of the true absorption rate. Indeed, a recent study with β-lactams found that in some cases luminal disappearance was much greater than absorption because some compounds can undergo substantial accumulation in the gut wall (Sugawara et al., 1990). BBMV have also been widely utilized in attempts to elucidate specific processes mediating peptide transport. While transcellular transport involves apical uptake and basolateral efflux, most studies have focused on peptide uptake by BBMV and very little is known about basolateral efflux. Only recently has there been some indication that basolateral efflux of dipeptides may involve a pH dependent carrier-mediated process although more studies will be required to fully characterize the role of this transporter in peptide absorption in vivo (Dyer et al., 1990).

An additional limitation of BBMV studies is that the peak-overshoot phenomenon, indicative of active transport, has not been shown as clearly for the peptide transporter (Hori et al., 1988) as is the case for amino acid and glucose transporters (Hopfer et al., 1976).

An earlier study demonstrated carrier-mediated transport of cephalexin in rat jejunum and determined a concentration for half-maximum transepithelial transport, K_t, of approx. 2 mM (Yamashita et al., 1984, 1986). That study showed the involvement of a carrier, presumably the di-/tripeptide transporter on the transport of cephalexin across rat jejunum at pH 7.4. In the present study, we investigated the transepithelial transport of cephalexin and benzylpenicillin in different segments of rabbit intestinal mucosa mounted in Ussing chambers. These results enabled us to: (a) compare cephalexin and benzylpenicillin transport across rat vs rabbit intestine; (b) compare transport across different intestinal segments; and (c) determine the role of a pH gradient on transepithelial transport of cephalexin and benzylpenicillin. Integrity and viability of intestinal tissues were assessed by measuring electrical parameters and transepithelial flux of the paracellular flux marker, mannitol.

Materials and Methods

Materials

D-[^{3}H]Cephalexin (4.1 Ci/mmol) was synthesized by the Radiochemistry Department (SmithKline Beecham Pharmaceuticals, King of Prussia, PA). D-Cephalexin was provided by Eli Lilly and Co. (Indianapolis, IN). Ouabain, citric

acid, mannitol, (α-D-glucose, prostaglandin E_1 (PGE_1), amiloride, 2-(*N*-morpholino)ethanesulfonic acid (Mes) and Hepes were purchased from Sigma Chemical Co. (St. Louis, MO). [^{14}C]Mannitol (49.3 mCi/mmol) and [*phenyl*-^{3}H]benzylpenicillin (34.9 Ci/mmol) were purchased from New England Nuclear Products (Boston, MA).

Methods

Preparation of tissue segments

Segments of jejunum, ileum, and distal colon from male New Zealand White rabbits (2–3 kg) were opened along the mesenteric border and rinsed with a bicarbonate-Ringer solution containing (in mM): Na^+, 141; K^+, 5; Ca^{2+}, 1.2; Mg^{2+}, 1.2; Cl^-, 122; HCO_3^-, 25; HPO_4^{2-}, 1.6; and $H_2PO_4^-$, 0.4. Intestinal mucosal segments were stripped of their underlying muscle as described previously (Smith et al., 1988) and mounted in Ussing chambers with a 3 cm^2 exposed surface area. Prior to adding test agents, tissues were equilibrated for 45–60 min with bicarbonate-Ringer solution containing 10 mM mannitol (in the mucosal bathing solution) and 2 mM mannitol plus 8 mM glucose (in the serosal bathing solution). Bathing solutions (12.5 ml) were adjusted to the appropriate pH and recirculated with O_2/CO_2 (95%/5%). The temperature of bathing solutions was controlled with water-jacketed reservoirs connected to recirculating water baths.

Tissue integrity and viability

To ensure that the fluxes of cephalexin across intestinal segments did not result from tissue damage, tissue integrity was monitored throughout experiments in two ways: (a) the electrical conductance (G_t) and (b) flux of the paracellular flux marker, mannitol (Dawson, 1977; Laker et al., 1982; Marks et al., 1991). Viability of jejunal, ileal and colonic tissues was assessed by measuring short-circuit current (I_{sc}) throughout the experiments and from the changes in I_{sc} resulting from addition of glucose (10 mM) to the luminal bathing solutions of jejunum or ileum or PGE_1 (10 μM) to the serosal bathing solution of distal colon at the end of flux measurements.

Transport studies

Transport experiments were carried out at 37°C in HCO_3^--Ringer using D-[^{3}H]cephalexin and [^{3}H]benzylpenicillin. To determine the effect of pH on transport, luminal bathing solutions consisted of HCO_3-Ringer containing 25 mM citric acid (pH 5–5.5), Mes (pH 6–6.5) or Hepes (pH 7–7.4). When the pH of the bathing solution was initially 7.4, gassing with O_2/CO_2 decreased this pH to approx. 7.1. On the other hand, if the initial pH of the bathing solution was 5.5, gassing increased this pH to 5.8 almost immediately. After this initial adjustment the pH values of both solutions (regardless of the initial gradient) remained constant to 230 min. This time exceeded the duration of our experiments. To determine transmucosal transport of cephalexin, both bathing solutions received unlabeled cephalexin (0.1 mM) and the donor side received 5 μCi of [^{3}H]cephalexin plus 5 μCi of [^{14}C]mannitol. At specified times, 100 μl samples were taken from the donor side and 1 ml samples from the receiver side. Samples taken from the receiver side were replaced with an equal volume of the appropriate bathing solution whereas samples from the donor side were not replaced. Radioactivity was determined in a 4640 Packard Tri-Carb Liquid Scintillation Spectrometer (Packard Inst., Sterling, VA). For cephalexin, dpm were either divided by dpm/nmol in order to determine the amount of cephalexin transported or divided by the amount of radioactivity applied to the donor chamber and then multiplied by 100 to obtain the percent transport. For mannitol all results are presented as percent transport. Quantification of transport was performed on the basis of the amount of radioactivity appearing in the receiver chamber.

Data analysis

All values are presented as mean and standard error of values from replicate tissues (2–4) from at least three rabbits. Differences between two means were tested using the two-tailed Student's *t*-test for independent samples (Schefler, 1979).

When comparing more than two means a one-way analysis of variance was used at first and then statistically significant differences identified by using a multiple-range test (Schefler, 1979). In all cases a significance level of 5% ($P < 0.05$) was required to reject the null hypothesis.

Results

Integrity of the intestinal preparations was assessed through measurements of transepithelial conductance (G_t) and unidirectional fluxes of mannitol. In rabbit ileum (Fig. 1A) and jejunum (data not shown), G_t was ~ 25 mS/cm^2 and remained constant over the time course of the experiments. These values are in agreement with those reported in previous studies (Field et al., 1971; Dawson, 1977). Mannitol fluxes display a 15–30 min lag time and are linear for the remainder of the flux period (Fig. 1B). In rabbit ileum, mannitol fluxes were 0.18 ± 0.03 and $0.17 \pm 0.08\%$ h^{-1} 3 cm^{-2} (six tissues from three animals) in the m-to-s and s-to-m direction, respectively. With the concentration of mannitol present in the mucosal (10 mM) and serosal (2 mM) bathing solutions, the fluxes of mannitol are 75 and 15 nmol h^{-1} cm^{-2}, respectively. These results are similar to those reported previously (Marks et al., 1991). The m-to-s and s-to-m mannitol fluxes in rabbit jejunum were $0.24 \pm 0.06\%$ h^{-1} 3 cm^{-2} (six tissues from three animals) and $0.18 \pm 0.03\%$ h^{-1} 3 cm^{-2} (six tissues from three animals), respectively (Fig. 1B). In rabbit distal colon, G_t averaged 4 mS/cm^2 and was constant over the time course of the experiments (Fig. 1A). Mannitol fluxes in this segment were $0.039 \pm 0.003\%$ h^{-1} 3 cm^{-2} (six tissues from three animals) (m-to-s) and $0.054 \pm 0.009\%$ h^{-1} 3 cm^{-2} (six tissues from three animals) (s-to-m).

The I_{sc} response of tissues at the end of experiments was used to assess viability. I_{sc} of ileal tissues remained relatively constant throughout the experiments and increased following addition of glucose to the mucosal bathing solution at the end of the flux period (Table 1). The response of the jejunal tissues after the addition of glucose (ΔI_{sc}) was 2.22 ± 0.15 μEq h^{-1} cm^{-2} ($n = 6$), which is identical to that observed with ileal tissues (ΔI_{sc} 2.22 ± 0.5 μEq h^{-1} cm^{-2}; $n = 6$). Colonic tissues also exhibited a pronounced increase in I_{sc} after the addition of PGE_1 to the serosal bathing solution (Table 1). These results are in agreement with previous studies (Field et al., 1971; Heintze et al., 1983) and indicate that active ion transport processes were not compromised over the time course or conditions of the experiments.

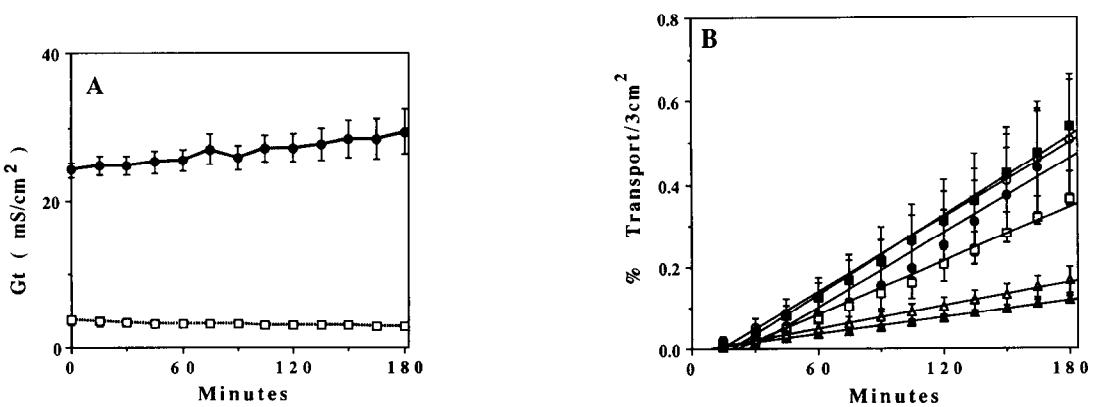

Fig. 1. (A) Time course of transepithelial conductance in rabbit ileum (closed circles) or rabbit distal colon (open boxes). Results are means $\pm$ 1 SE for six tissues from three animals. Zero time is 50 min after mounting tissues in vitro. (B) Time course of mannitol fluxes across rabbit ileum (circles: closed, m-to-s; open, s-to-m), rabbit jejunum (squares: closed, m-to-s; open, s-to-m) and rabbit distal colon (triangles: closed, m-to-s; open, s-to-m). Results are means $\pm$ 1 SE for six tissues from three animals. [^{14}C]Mannitol was added at zero min (50 min after mounting the tissue in the chambers).

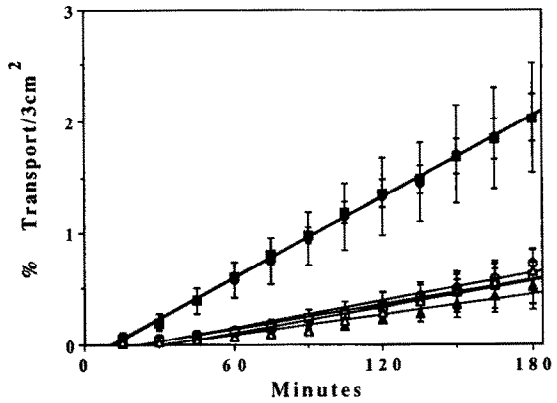

Fig. 2. Time course of cephalexin transport across rabbit ileum (circles: closed, m-to-s; open, s-to-m), jejunum (squares: closed, m-to-s; open, s-to-m), and distal colon (triangles: closed, m-to-s; open s-to-m). Results are means ± 1 SE (jejunum, $n = 3$; ileum, $n = 6$; distal colon, $n = 6$). Zero time is 50 min after mounting tissues in vitro.

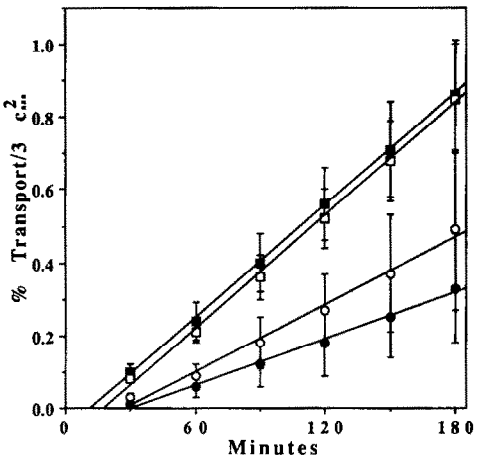

Fig. 3. Time course of benzylpenicillin (squares: closed, m-to-s; open, s-to-m) and mannitol (circles: open, m-to-s; closed, s-to-m) fluxes across rabbit jejunum. Results are means ± 1 SE for three animals.

Unidirectional cephalexin fluxes in these intestinal segments with both bathing solutions at pH 7.4 are presented in Fig. 2. In jejunum, ileum and distal colon, the s-to-m fluxes were 0.21 ± 0.04, 0.24 ± 0.02 and 0.21 ± 0.04% h^{-1} 3 cm^{-2}, respectively. In jejunum and ileum, the m-to-s fluxes of cephalexin were 0.66 ± 0.12 and 0.66 ± 0.30% h^{-1} 3 cm^{-2}, respectively, which is 3-times greater than the s-to-m fluxes in these segments while the m-to-s flux in distal colon was 0.18 ± 0.12% h^{-1} 3 cm^{-2}, which is identical to the s-to-m flux.

Unidirectional benzylpenicillin fluxes in rabbit jejunum with both bathing solutions at pH 7.4 are presented in Fig. 3. In contrast to the results with cephalexin, benzylpenicillin fluxes were identical in both directions: m-to-s, 0.27 ± 0.05% h^{-1} 3 cm^{-2}; s-to-m, 0.28 ± 0.05% h^{-1} 3 cm^{-2}. These

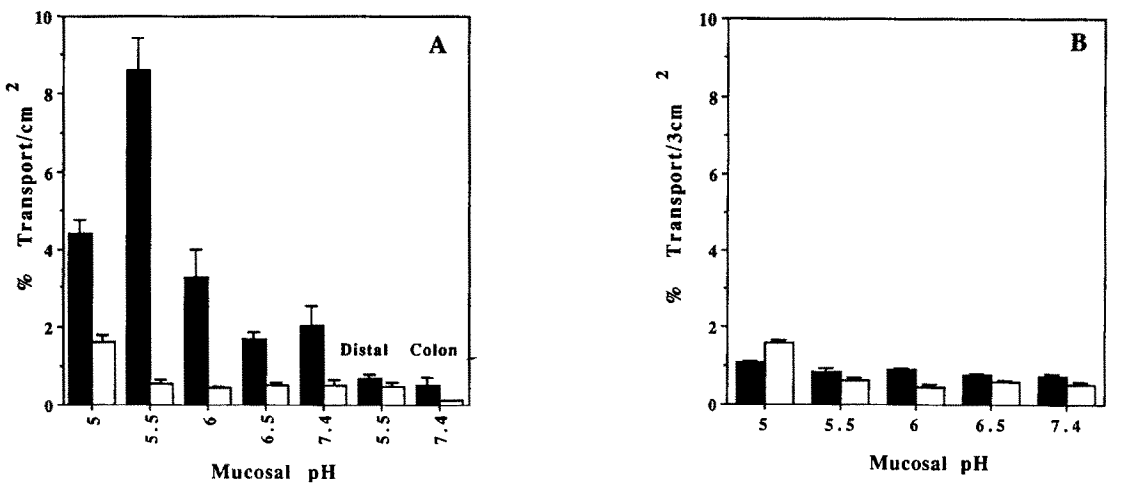

Fig. 4. pH dependence (mucosal pH varies between 5 and 7.4 with serosal pH constant at pH 7.4) of mucosal-to-serosal (A) and serosal-to-mucosal (B) cephalexin (solid bars) and mannitol (open bars) fluxes across rabbit ileum and distal colon. Results are means ± 1 SE for four animals.

fluxes were comparable to s-to-m flux of cephalexin ($0.21 \pm 0.04\%$ h^{-1} 3 cm^{-2}). Reducing the mucosal bathing solution pH from 7.4 to 5.5 did not alter the fluxes of benzylpenicillin. Since there was no evidence for a carrier mediated transport of benzylpenicillin, subsequent studies were conducted with cephalexin only.

Because the results obtained with jejunum and ileum are very similar, additional studies were performed only with ileal tissues. When the luminal bathing solution pH was reduced from 7.4 to 5.5, m-to-s flux of cephalexin in rabbit ileum increased from 0.66 ± 0.09 to $2.85 \pm 0.12\%$ h^{-1} 3 cm^{-2} while the s-to-m flux of cephalexin was not significantly altered (Fig. 4A and B). However, in rabbit distal colon, reduction of bathing solution pH did not alter m-to-s transport of cephalexin. The m-to-s fluxes were 0.17 ± 0.04 and $0.21 \pm 0.04\%$ h^{-1} 3 cm^{-2} when pH of the mucosal solution was 7.4 and 5.5, respectively. Although the amount of cephalexin associated with the tissues was not determined in this study, it appears that tissue accumulation was low. In the absence of an imposed pH gradient $2 \pm 0.3\%$ of that cephalexin applied to the mucosal (donor) side 0.1 mM was transported to the serosal receiver side in 3 h, and $97.8 \pm 3.5\%$ remained in the donor side. When the pH of the luminal bathing solution was 5.5 and that of the serosal bathing solution 7.4, 8.5 ± 0.4 and $91.4 \pm 2.4\%$ were found in the receiver and donor chambers, respectively.

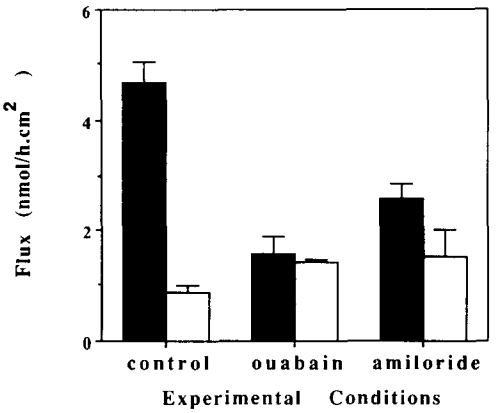

Fig. 5. Effects of serosal ouabain (0.1 mM) or luminal amiloride (1 mM) on cephalexin (solid) and mannitol (open) fluxes in rabbit ileum with pH 7.4 in both bathing solutions. Results are means $\pm$ 1 SE for three animals.

Adjusting the pH of the luminal solution over the range of 7.4–5.5 had no effect on tissue viability as assessed by baseline I_{sc} values of ileal tissues during the experiments or the increase in I_{sc} that followed the addition of glucose to the luminal bathing solution at the end of the flux studies (Table 1). These changes in pH also did not alter tissue integrity as assessed by the basal G_t (not shown) and mannitol fluxes (Fig. 4A and B). Similarly, reducing the pH from 7.4 to 5.5 had no effect on colonic viability since the basal I_{sc} and the increase in I_{sc} observed after adding PGE_1 to the serosal bathing solution were unaltered (Table 1). The flux of mannitol in the colon at pH 5.5 was $0.19 \pm 0.05\%$ h^{-1} 3 cm^{-2} which is approx. 5-fold greater than mannitol at pH 7.4.

Addition of 0.1 mM ouabain to the serosal bathing solution of ileal mucosa inhibited the m-to-s flux of cephalexin by 70% (Fig. 5) and reduced I_{sc} from 2.5 ± 0.34 to 0 μEq h^{-1} cm^{-2} indicating that active ion transport was abolished. In addition, the changes in I_{sc} elicited by mucosal addition of glucose were reduced from 2.2 ± 0.5 to 0.3 ± 0.05 μEq h^{-1} cm^{-2} (Table 1) consistent with dissipation of the sodium gradient responsible for sodium glucose uptake at the apical mem-

TABLE 1

Maximal change in I_{sc} (μEq h^{-1} cm^{-2}) occurring within 10 min following addition of glucose (10 mM) to the mucosal bathing solution of rabbit ileum or, serosal addition of PGE_1 (10 μM) to rabbit distal colon following transport studies

Mucosal solution	Tissue response (ΔI_{sc}) (μEq h^{-1} cm^{-2})	
	Ileum	Distal colon
KBR [b]/pH 7.4 (control)	2.2 ± 0.5	2.7 ± 0.3
KBR/pH 5.5	2.7 ± 0.3	2.1 ± 0.3
Amiloride/pH 7.4	2.1 ± 0.3	ND
Ouabain/pH 7.4	0.3 ± 0.05 [a]	ND

Values shown are mean ($n = 6$–12) plus or minus standard error of the mean (SE); [a] $P < 0.01$ compared to controls; ND, not determined.

[b] Bicarbonate-Ringer solution described in Materials and Methods.

brane. Addition of 0.1 mM amiloride to the mucosal bathing solution, resulted in a 40% inhibition of cephalexin flux (Fig. 5) with no change in the basal I_{sc} (4.2 ± 0.40 μEq h^{-1} cm^{-2}) or the ΔI_{sc} (2.1 ± 0.3 μEq h^{-1} cm^{-2}) elicited by mucosal addition of glucose (Table 1). Mucosal-to-serosal (Fig. 5) and serosal-to-mucosal fluxes of mannitol were not altered by ouabain or amiloride (not shown).

Discussion

In prior studies, it was demonstrated that cephalexin is transported by a carrier mediated process in Caco 2 cells and rat intestinal tissues (Yamashita et al., 1984, 1986; Danzig and Bergin, 1990). In addition, in Caco 2 cells, cephalexin uptake was shown to be pH dependent. These findings together with results from uptake studies in intestinal and renal brush border membrane vesicles have been cited as support for transport of cephalexin by the di-/tripeptide transporter (Ganapathy and Leibach, 1985; Tiruppathi et al., 1991). In vesicle studies, benzylpenicillin has been shown to interact with the di-/tripeptide transporter and it has been used as a photoaffinity label for isolation of a 127 kDa protein (Kramer et al., 1990a,b). In the present study, transepithelial transport of both cephalexin and benzylpenicillin were compared to determine whether both these molecules can be employed as substrates for studying the intestinal peptide transporter. The results presented in Figs 2 and 3 show that cephalexin but not benzylpenicillin is a good substrate for the peptide transporter. Thus, although benzylpenicillin interacts with the di-/tripeptide transporter, this interaction does not result in enhanced transepithelial transport. Whether this is a unique feature of benzylpenicillin or will also apply to other molecules will require further investigation. These results emphasize the necessity of measuring transepithelial transport and not just competition, uptake or disappearance from the lumen to determine whether interaction with the transporter will result in enhanced absorption.

From these studies, a species comparison of transport of cephalexin and benzylpenicillin between rat and rabbit can be made. In both rat and rabbit, transport of cephalexin in small intestinal tissues is carrier-mediated. The half-maximal transport rate for the mucosal to serosal flux of cephalexin is ~ 2 mM in both rat jejunum and rabbit ileum (Yamashita et al., 1986; Gochoco et al., 1991). The passive permeabilities in the two species, calculated from the serosal to mucosal fluxes, are comparable (rat = 0.011 cm/h (Yamashita et al., 1986) and rabbit = 0.007 cm/h). Mucosal to serosal fluxes of benzylpenicillin are similar to serosal to mucosal fluxes in the rat and rabbit (Fig. 2, Ryan and Smith, 1989). Prior studies with rat small intestine proposed a concentration dependent process for benzylpenicillin absorption although this was not confirmed in subsequent studies (Stewart and Jackson, 1981; Ryan and Smith, 1989).

Carrier-mediated transport processes for nutrients are predominantly located in the small intestine. To assess whether this is also true for the di-/tripeptide transporter, transport of cephalexin in both small and large intestinal segments was determined. Similar to what has been observed for absorption of other nutrients, carrier-mediated transport of cephalexin was confined to the small intestine. Within the small intestine, transport rates were similar in both the jejunum and ileum.

Prior studies have examined the mechanisms involved in transepithelial transport of cephalexin, a substrate for the intestinal peptide transporter which is stable to intestinal peptidases and exhibits oral bioavailability of 90% (Finkelstein et al., 1978). The Ussing technique provides a method for assessing and monitoring viability and integrity of intestinal tissues and can also provide information regarding physiological changes in ion transport resulting from the molecules being studied (Field et al., 1971; Smith and Field, 1980; Marks et al., 1991).

In the present studies, there were no significant changes in I_{sc} or G_t elicited by cephalexin. In addition, cephalexin did not alter tissue permeability as assessed by mannitol fluxes or tissue viability determined from the response to absorptive or secretory stimuli. Tissues were continu-

ously short-circuited, thereby eliminating any transepithelial electrical gradient which could be a driving force for transepithelial movement of charged molecules. Furthermore, the solutions bathing both tissue surfaces contained the same concentration of cephalexin (i.e., no transepithelial concentration gradient). In rabbit ileum and jejunum, with identical bathing solutions on both sides of the tissue and a pH of 7.4, the m-to-s flux of cephalexin is ~ 3-fold greater than the s-to-m flux, consistent with carrier-mediated transport of cephalexin in the absorptive direction. In rabbit distal colon under identical conditions there were no differences in the unidirectional fluxes suggesting that a similar carrier-mediated mechanism is not present in this segment.

According to the model for di-/tripeptide transport proposed by Leibach and Ganapathy (1985), cephalexin uptake at the apical membrane is carrier-mediated and driven in part by a proton gradient across this membrane. The presence of a microclimate pH which is lower than the bulk luminal pH in vivo has been reported previously (Lucas, 1983). In vitro, the microclimate pH gradient may be less than that encountered in vivo due to the more efficient mixing provided by the technique employed (Hidalgo et al., 1991). Thus, the magnitude of the absorptive flux determined in vitro with pH 7.4 buffer in the luminal bathing solution (Fig. 2 and Yamashita et al., 1984, 1986) may be an underestimation of the flux occurring in vivo. This possibility was investigated by reducing the pH of the luminal bathing solution from 7.4 to 5.5. This maneuver increased the m-to-s flux of cephalexin without altering either the m-to-s or the s-to-m flux of mannitol. These results are consistent with a proton-dependent carrier-mediated uptake process for cephalexin and suggest that in vivo the magnitude of the absorptive flux will be greater than that seen in vitro with pH 7.4 buffer in the luminal bathing solution. Whether this proton dependence is due to a change in carrier conformation or a direct proton-cephalexin cotransport will require further studies. It does not appear that the increase in cephalexin absorption at pH 5.5 is due to an effect on cephalexin ionization to alter its permeability characteristics, since reducing the pH from 7.4 to 5.5 in the distal colon had no effect on cephalexin absorption.

The model for peptide transport also predicts that inhibition of the basolateral Na^+/K^+-ATPase or the apical Na^+/H^+-exchanger should reduce the m-to-s flux of cephalexin by dissipating the microclimate pH. From Fig. 5 it can be seen that the m-to-s flux of cephalexin is inhibited by serosal addition of the Na^+/K^+-ATPase inhibitor, ouabain and by luminal addition of the Na^+/H^+-exchange inhibitor, amiloride (Kinsella and Aronson, 1981) supporting the proposal that a pH gradient across the apical membrane is required for transepithelial transport.

This study provides direct evidence that a proton-dependent, carrier-mediated uptake mechanism, results in increased transepithelial transport of cephalexin. The mechanism by which cephalexin exits across the basolateral membrane cannot be determined from the present studies, but may involve a carrier-mediated process as proposed by Beechy and co-workers (1990).

This transport process is confined to the small intestine and is present in both rat and rabbit. Results from this study also emphasize the need to determine transport of molecules since the β-lactam, benzylpenicillin, competes with cephalexin uptake but this uptake does not result in transepithelial transport. This conclusion is supported by a recent study by Kramer et al. (1992). After reconstituting a 127 kDa binding protein (presumably the peptide transporter or a component of it) into liposomes they found that both D- and L-cephalexin bound to the binding protein. However, only the D-isomer was taken up into the liposomes. While that difference is due to stereoselective uptake by the carrier, it constitutes an example in which binding to the carrier does not result in uptake.

Acknowledgements

The authors would like to thank Drs Richard Heys and Steve Senderoff, Department of Synthetic Chemistry, for synthesis of radiolabeled cephalexin; Drs Richard Kirsh and Doris Wall for helpful criticisms and Marge Glennon and Pat Miller for expert secretarial help.

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