Cimetidine Transport in Brush-Border Membrane Vesicles from Rat Small Intestine

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ABSTRACT

In previous studies, sulfoxide metabolite was observed in animal and human intestinal perfusions of cimetidine and other H2-antagonists. A sequence of follow-up studies is ongoing to assess the intestinal contributions of drug metabolism and drug and metabolite transport to variable drug absorption. An evaluation of these contributions to absorption variability is carried out in isolated fractions of the absorptive cells to uncouple the processes involved. This paper is presented on the drug entry step from a study on [3H]cimetidine uptake into isolated brush-border membrane vesicles from rat small intestine. A saturable component for cimetidine uptake was characterized by a Vmax and Km (mean ± S.E.M.) of 6.1 ± 1.5 nmol/30s/mg protein and 8.4 ± 2.0 mM, respectively. Initial binding, and possibly intravesicular uptake, was inhibited by other cationic compounds including ranitidine, procainamide, imipramine, erythromycin, and cysteamine but not by TEA or by the organic anion, probenecid. Initial uptake was not inhibited by amino acids methionine, cysteine or histidine, by the metabolite cimetidine sulfoxide, or by inhibitors of cimetidine sulfoxidation, methimazole, and disulfonic acid. Equilibrium uptake was inhibited by ranitidine, procainamide, and cysteamine but not by erythromycin or imipramine. Initial cimetidine uptake was stimulated by an outwardly directed H+ gradient, and efflux was enhanced by an inwardly directed H+ gradient. Collapse of the H+ gradient as well as voltage-clamping potential difference to zero significantly reduced initial cimetidine uptake. The data is supportive of both a cimetidine/H+ exchange mechanism and a driving-force contribution from an inside negative proton or cation diffusion potential.

Cimetidine is a histamine H2-receptor antagonist drug used to treat duodenal ulcers and gastric acid hypersecretion. Although the drug is well absorbed after oral administration, substantial absorption variability has been reported as a function of oral administration conditions (Lipsy et al., 1990). From a study on the absorption of cimetidine through an everted sac preparation in rat small intestine, the authors reported the uncovering of an “active” cimetidine absorption component dominated by “passive” permeation at higher concentrations (Barber et al., 1979).

Secretion of cimetidine in the kidney has been demonstrated in whole animals (Weiner and Roth, 1981; McKinney et al., 1981; McKinney and Speeg, 1982; Cacini et al., 1982; Rennick et al., 1984). The development of isolated renal and other epithelial brush-border membrane vesicles (BBMV) and basolateral membrane vesicles offers an experimental system that provides more detailed information on cimetidine transport processes. In the brush-border membrane of rabbit kidney, active transport of cimetidine was observed via an organic cation transport system using a proton gradient as the driving force (Gisclon et al., 1987). Cimetidine transport in isolated BBMV from bovine choroid plexus showed both saturable and nonsaturable transport components (Whittico et al., 1990). Carrier-mediated transport of cimetidine has also been reported in isolated rat hepatocytes (Nakamura et al., 1994). However, the pathways for the small intestinal brush-border membrane transport of cimetidine have not yet been delineated.

The goals of this study were to 1) determine the transport pathways for cimetidine in rat intestinal BBMV, 2) identify the driving forces for cimetidine transport, and 3) evaluate the effects of other drugs and nutrients on cimetidine uptake.

Experimental Procedures

Materials. Cimetidine and all other chemicals were purchased from Sigma Chemical Co. (St. Louis, MO) unless otherwise stated. 1-Amino-2-methyl-2-propanethiol and 2-(ethylthio)ethyamine were obtained from Aldrich (Milwaukee, WI). d-[1-3H]Glucose (specific activity 15.5 Ci/mmol) was obtained from New England Nuclear (Boston, MA). [N-methyl-3H]cimetidine (specific activity 11.3 Ci/mmol) was purchased from Amersham (Arlington Heights, IL). Ci...
metidine sulfoxide was generously provided by SmithKline Beecham Pharmaceuticals (King of Prussia, PA).

Preparation of BBMV. Intestinal BBMV were prepared from the jejunum of 200- to 250-g male rats (Sprague-Dawley; Charles River Breeding Laboratories, Pearl River, NY) by a calcium precipitation method as described by Yuasa et al. (1993). Vesicles were prepared the day before use and stored at 4°C overnight. Protein concentrations were determined by the method of Bradford (1976) using a Bio-Rad protein assay kit (Bio-Rad, Richmond, CA) with bovine serum albumin as a standard. Enzyme activities were measured using the following assays. Alkaline phosphatase activities were monitored using a commercially available ALP kit (Sigma Diagnostics, St. Louis, MO). Na⁺,K⁺-ATPase activities were performed according to Jørgensen and Skou (1969) with phosphate determined by the method of Fiske and Subbarow (1925). Arylesterase activity was determined using the method of Sheppard and Hubscher (1969). Enrichment was calculated by comparing the ratio of specific enzyme activity in the BBMV to that of homogenate.

Uptake Experiments. Uptake experiments were carried out at 25°C ± 3°C by a rapid filtration technique using a Millipore filtration apparatus (Millipore Corp., Bedford, MA). Typically, 40 μl of uptake mixture was rapidly mixed with 10 μl of membrane vesicles (0.07–0.1 mg protein). After incubation for a specific time period, the reactions were stopped by adding 4 ml of ice-cold buffer containing 100 mM mannitol, 100 mM potassium chloride, and 10 mM HEPES, pH 7.5. The stopped reaction mixture was filtered under vacuum of 20 mm Hg through a 0.3 μm prewetted nitrocellulose membrane (Type PHWP, Millipore) and washed four times with 4 ml of ice-cold stop buffer. The radioactivity retained on the filter was determined using a Beckman LS 6000 SC scintillation counter (Beckman Instruments, Inc., San Jose, CA). All uptake measurements were corrected for nonspecific filter binding.

D-glucose uptake was measured using a loading buffer containing 100 mM KCl, 100 mM mannitol, and 10 mM HEPES, adjusted to pH 7.5 with Tris buffer. The uptake solution contained 100 μM D-glucose traced with 2 μCi/ml [3H]-d-glucose in buffer composed of 100 mM NaCl, 100 mM mannitol, and 10 mM HEPES/Tris (pH 7.5). Cimetidine uptake was determined using 50 μM cold cimetidine traced with 2 μCi/ml [3H]-cimetidine to provide a radioactive concentration of 40 μCi/mmol. Detailed composition of loading and uptake buffers is described in the legends for figures and tables. Cimetidine efflux was examined by loading vesicles with 500 μM cold cimetidine in mannitol/2%(w/v) morpholino)ethanesulfonic acid (MES)-Tris buffer at pH 7.5 traced with 5 μCi/ml [3H]cimetidine to provide an efflux study-radioactive concentration of 10 μCi/mmol. Cimetidine appearance in the incubation buffer (pH 7.5 and pH 6.0) was monitored at predetermined time points. Osmolality experiments were performed by incubating the vesicles in uptake buffers with varying amounts of sucrose.

Cimetidine Metabolism. The extent of metabolism of cimetidine in the vesicle preparation was determined using a method described previously (Lu et al., 1998). Briefly, 1 mg of rat intestinal BBMV was mixed with the reaction medium containing 50 mM potassium phosphate (pH 7.4), 0.5 mM NADP⁺, 2.0 mM glucose-6-phosphate, and 2 IU glucose-6-phosphate dehydrogenase. The reaction mixture was preincubated for 5 min at 37°C and started by the addition of 0.1 mM cimetidine. The reactions were stopped by adding 200 μl of 10% trichloroacetic acid. After centrifugation, the supernatant was adjusted to pH 7.0 with 200 μl of 0.5 M sodium phosphate. Cimetidine and cimetidine sulfoxide were extracted using a C18 solid phase extraction column (Alltech Associates, Inc., Deerfield, IL) and analyzed using HPLC with codeine as an internal standard (Larsson et al., 1982).

Results

Vesicle Characterization and Cimetidine Uptake. The specific activity of the brush-border marker enzyme, alkaline phosphatase, in the vesicle preparation was increased over that of crude intestinal homogenate with an enrichment factor of 13.7 ± 1.0. Na⁺,K⁺-ATPase activity was not enhanced in the preparation, indicating minimal contamination with basolateral membranes (enrichment factor = 0.84) and contamination by the microsomal fraction is negligible (enrichment factor of arylesterase was 0.03). A characteristic overshoot phenomenon for the uptake of D-glucose was evident in the presence of an inwardly directed sodium gradient, documenting the presence of functional luminal membrane vesicles.

In the absence of pH and other ion gradients (lower curve ▲ and inset in Fig. 1), cimetidine uptake increased rapidly in the first minute and approximately 50% of equilibrium cimetidine uptake was achieved after 10 min of incubation. Cimetidine uptake then gradually increased over 4 h. Uptake values at 3 and 4 h were not statistically different, so 4-h incubation was used for equilibrium studies. Incubation of cimetidine with BBMV for 4 h did not generate significant cimetidine sulfoxide, indicating that the appearance of metabolite observed in the jejunum in vivo did not occur at the rat brush-border membrane (data not shown). Cimetidine uptake was linear up to 30 s, so data at 30 s was subsequently used in initial uptake studies. Vesicle uptake of cimetidine decreased in a linear manner with increasing medium osmolality generated by adding increasing concentrations of sucrose, suggesting that cimetidine uptake is osmolality-sensitive and corresponds to intravesicular transport (Fig. 2). Although intravesicular volume could not be calculated under these conditions, extrapolation to infinite osmolality suggests that cimetidine binding to vesicle membranes may account for as much as 60% of equilibrium uptake.

Fig. 1. Effect of an outwardly directed H⁺ gradient on 50 μM cimetidine (40 μCi/mmol) uptake in rat intestinal BBMV. Membrane vesicles were preloaded with 100 mM MES, 25 mM Tris (pH 5.7); or 25 mM MES, 75 mM HEPES, 25 mM Tris (pH 6.5); or 75 mM HEPES, 50 mM Tris (pH 7.5) containing 200 mM mannitol. Uptake buffer contained 75 mM HEPES, 50 mM Tris, 200 mM mannitol (pH 7.5). Concentration of cimetidine was 50 μM. Data are the mean ± S.E.M. of triplicate uptake samples measured in three separate membrane preparations, except at intravesicular pH 6.5, where data were obtained from triplicate uptake measurement of one membrane preparation.
Outwardly Directed H\textsuperscript{+} Gradient and Membrane Potential Effects on Cimetidine Uptake. Figure 1 demonstrates the effect of intravesicular pH on the uptake of cimetidine by BBMV in a Na\textsuperscript{+} and K\textsuperscript{+}-free medium at room temperature. The intravesicular pH was varied at 5.7, 6.5, and 7.5 and the extravesicular pH was fixed at 7.5. As shown, cimetidine uptake was enhanced by the effect of an outwardly directed H\textsuperscript{+} gradient. A significant overshoot phenomenon was observed at 2 min when the vesicles were preloaded with pH 5.7 buffer. This overshoot was very sensitive to intravesicular pH as changes of ±0.3 U from a buffer pH of 5.7 substantially diminished the overshoot (data not shown). At the peak time of overshoot, the intravesicular concentration of cimetidine was about twice the equilibrium value.

The role of an inside negative diffusion potential was further examined by preloading BBMV with 100 mM potassium gluconate. The suspension of K\textsuperscript{+}-loaded vesicles was diluted with a K\textsuperscript{+}-free incubation medium that contained valinomycin (10 \(\mu\)g/mg protein). Because gluconate ion is less permeable than K\textsuperscript{+} (Saitoh et al., 1988), a K\textsuperscript{+} gradient from in to out was used to generate an intravesicular negative state (Takuwa et al., 1985). As illustrated in Fig. 4, in comparison with drug uptake in the control study, initial cimetidine uptake in the absence of an H\textsuperscript{+} gradient was enhanced when an inside negative membrane potential was imposed. Although experiments were only conducted in two vesicle preparations, this result agrees with an unpublished study by A. Tsuji et al. from Kanazawa University (A. Tsuji, A. Kadawaki, T. Terasaki and G.L. Amidon, personal communication).

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The effect of transmembrane potential difference on cimetidine uptake was also examined by evaluating the impact of voltage clamping potential difference to zero in the presence and absence of an outwardly directed H\textsuperscript{+} gradient. This was carried out by replacing 200 mM mannitol with 100 mM KCl in preloading and incubation buffers and including 35 \(\mu\)M valinomycin in voltage-clamp studies. Although voltage
Clamping is observed to significantly reduce cimetidine uptake in the presence of a proton gradient, the reduction is not as great or as rapid as that observed with FCCP (Fig. 5). In the absence of a proton gradient, cimetidine uptake is further reduced and voltage clamping potential difference to zero or the addition of FCCP is confirmed to have no effect (Fig. 6).

**Effect of an Inwardly Directed Na\(^+\) Gradient on Cimetidine Uptake.** It is noteworthy that the initial uptake rate of cimetidine has been substantially reduced in potential difference experiments using buffer solutions in which potassium salts replaced mannitol (Figs. 3 and 4 versus Fig. 1). A similar depression of cimetidine uptake was observed when mannitol was replaced by equiosmolal concentrations of sodium chloride in uptake buffer solutions. Cimetidine uptake was 34% lower in the presence of an inwardly directed Na\(^+\) gradient as compared with control uptake under conditions in which this gradient was not imposed (data not shown). A similar effect has been reported for the uptake of the organic cation, guanidine, (Miyamoto et al., 1988) and the polyamine, putrescine, in rabbit intestinal BBMV (Milovic et al., 1995). Tsuji et al. also found that replacement of mannitol with equiosmol univalent cation-chloride salts substantially reduced cimetidine uptake in intestinal BBMV (A. Tsuji, A. Kadowaki, T. Terasaki and G.L. Amidon, personal communication).

**Concentration Dependence of Cimetidine Uptake in Rat Intestinal BBMV.** To distinguish between binding and actual uptake into BBMV, uptake was corrected by subtracting the values obtained under the same experimental conditions when vesicle integrity was destroyed by addition of 0.5% Triton X-100 (Murer et al., 1976). The effect of concentration on cimetidine uptake in the absence of an H\(^+\) gradient was investigated over the range of 25 \(\mu\)M to 40 \(\mu\)M cimetidine after a 30-s incubation. Figure 7 shows the concentration-dependent studies for cimetidine uptake at room temperature (25 ± 3°C) and at 4°C. The data illustrate that cimetidine uptake at 4°C was significantly less than that at room temperature. Uptake over this concentration range at room temperature was nonlinear, suggesting a mixed saturable/nonsaturable process. The kinetics of cimetidine uptake were characterized by the following equation:

\[
V = \frac{V_{\text{max}} \cdot C}{K_m + C} + K_d \cdot C
\]

where \(V\) represents the uptake rate, \(V_{\text{max}}\) is the maximal rate of uptake, \(K_m\) is the Michaelis constant, \(K_d\) is the rate constant for the nonsaturable uptake component, and \(C\) is the substrate concentration. The nonsaturable uptake rate con-
Eadie-Hofstee analysis did not provide resolvable kinetic parameters. However, after subtraction of cimetidine uptake at 4°C, parameters from nonlinear regression analysis indicated that a saturable component of cimetidine uptake represents 91% of total uptake over the initial 30 s in rat intestinal BBMV. Inclusion of a second parallel saturable component did not provide a good data fit with regression analysis.

**Cis Inhibition of Cimetidine Uptake.** The inhibition studies were carried out at initial time (30 s) and at equilibrium (4 h) in the presence and absence of an outwardly directed H⁺ gradient. Test inhibitors at 10 mM concentrations were added to the uptake solution. Equiosmolar concentrations of sucrose were used in place of inhibitors to determine whether extent of inhibition needed to be corrected for an osmotic effect. No significant decrease of cimetidine uptake was observed in the presence of sucrose at the concentration tested, indicating that 10 mM compounds added to the uptake solutions had no effect on vesicular volume.

As shown in Table 1, initial uptake of cimetidine was significantly inhibited by ranitidine, procainamide, imipramine, erythromycin, cysteamine, and by cimetidine itself. At equilibrium, ranitidine, procainamide, and cysteamine produced significant inhibition on cimetidine uptake. The amino acid, cysteine, significantly inhibited equilibrium uptake while failing to inhibit initial uptake of cimetidine.

**Effect of an Inwardly Directed H⁺ Gradient on Cimetidine Efflux.** Figure 8 demonstrates the effect of an inwardly directed proton gradient on the efflux of cimetidine from vesicles at room temperature in a single preparation. The percentage of cimetidine remaining in the vesicles after drug loading with vesicle interior pH at 7.5 is observed to be lower when the incubation medium is at pH 6.0 as compared

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**TABLE 1**

Inhibition effects on cimetidine rat intestinal brush-border vesicle uptake

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Presence of an outward H⁺ gradient</th>
<th>Absence of a H⁺ gradient</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>30 s</td>
<td>4 h</td>
</tr>
<tr>
<td><strong>10 mM</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>100°</td>
<td>100°</td>
</tr>
<tr>
<td>Sucrose</td>
<td>99.9 ± 3.3</td>
<td>95.2 ± 13.1</td>
</tr>
<tr>
<td>Cimetidine</td>
<td>55.1 ± 3.9*</td>
<td>48.8 ± 4.5**</td>
</tr>
<tr>
<td>Ranitidine</td>
<td>50.5 ± 13.8*</td>
<td>54.1 ± 12.8**</td>
</tr>
<tr>
<td>Cimetidine sulfoxide</td>
<td>96.9 ± 2.2</td>
<td>74.2 ± 3.4</td>
</tr>
<tr>
<td>Probencid</td>
<td>90.0 ± 2.7</td>
<td>74.7 ± 2.4</td>
</tr>
<tr>
<td>TEA</td>
<td>96.3 ± 7.6</td>
<td>89.8 ± 8.6</td>
</tr>
<tr>
<td>Procainamide</td>
<td>37.2 ± 2.3*</td>
<td>53.0 ± 9.8**</td>
</tr>
<tr>
<td>L-methionine</td>
<td>97.9 ± 3.3</td>
<td>102.8 ± 4.8</td>
</tr>
<tr>
<td>L-cysteine</td>
<td>93.7 ± 6.3</td>
<td>51.0 ± 2.1**</td>
</tr>
<tr>
<td>L-histidine</td>
<td>92.1 ± 8.4</td>
<td>84.0 ± 6.2</td>
</tr>
<tr>
<td>Methimazole</td>
<td>97.8 ± 14.4</td>
<td>88.5 ± 7.7</td>
</tr>
<tr>
<td>Imipramine</td>
<td>8.7 ± 5.3*</td>
<td>91.0 ± 16.1</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>35.4 ± 7.5*</td>
<td>82.7 ± 12.8</td>
</tr>
<tr>
<td>Cysteamine</td>
<td>52.0 ± 1.9*</td>
<td>30.1 ± 7.5*</td>
</tr>
<tr>
<td>1-Amino-2-methyl-2-propanethiol</td>
<td>40.3 ± 8.3*</td>
<td>39.8 ± 7.1*</td>
</tr>
<tr>
<td>2-(Ethylthio)ethylamine</td>
<td>47.4 ± 7.2*</td>
<td>86.2 ± 7.6</td>
</tr>
<tr>
<td>DIDS</td>
<td>68.8 ± 6.4</td>
<td>91.9 ± 7.1</td>
</tr>
</tbody>
</table>

Membrane vesicles were preloaded with 100 mM MES, 25 mM Tris (pH 5.7) or 75 mM HEPES, 50 mM Tris (pH 7.5) containing 200 mM mannitol. The uptake of 50 μM cimetidine was assayed in 75 mM HEPES, 50 mM Tris, 200 mM mannitol (pH 7.5) in the absence (control) and presence of the potential inhibitors. Data represent the mean ± S.E.M. of three separate membrane preparations.

- Data are the average of triplicate uptake measurement from one membrane preparation.
- Control values at 193.16 ± 16.712 pmol/mg protein/30 s represent 100% cimetidine uptake.
- Control values at 171.14 ± 6.477 pmol/mg protein/4 h represent 100% cimetidine uptake.
- Control values at 38.951 pmol/mg protein/30 s represent 100% cimetidine uptake.
- Control values at 142.103 pmol/mg protein/4 h represent 100% cimetidine uptake.
- ° p < .005; ** p < .05; *** p < .01.
with an incubation medium of pH 7.5. This is consistent with physiologic conditions in the rat jejunum where enterocyte cytosolic pH is considerably higher than the mucosal microclimate pH.

Discussion

This study was motivated by an observation on intestinal cimetidine metabolism (Hui et al., 1994). It was noted that the amount of cimetidine sulfoxide secreted into the intestinal lumen of the rat from in vivo jejunal perfusions of cimetidine was limited by cimetidine permeability (Piyapolrungroj, 1998). In these same studies, it was demonstrated that a reduction in cimetidine permeability occurred at high cimetidine concentrations, suggesting an involvement of a saturable process in cimetidine transport and a pH dependence not consistent with nonionic passive permeation of this weakly basic drug. The present results provide evidence for a saturable cimetidine transport component in the brush-border membrane isolated from rat small intestine.

The mechanism of cimetidine transport across jejunal brush-border membrane has not been previously detailed. The uptake of cimetidine in isolated renal and choroid plexus BBMV has been reported to include a carrier-mediated component. In both of these vesicle preparations, cimetidine uptake was significantly accelerated by an outwardly directed proton gradient, and an organic cation-H\(^+\) antiporter has been implicated (Takano et al., 1985; McKinney and Kunnemann, 1987; Gislon et al., 1987; Whittico et al., 1990).

In these studies, cimetidine uptake by rat intestinal BBMV was also observed to be stimulated by an outwardly directed H\(^+\) gradient. The presence of an organic cation-H\(^+\) antiport system in rabbit intestinal brush-border membrane was demonstrated by Miyamoto et al. (1988) for guanidine transport. However, a number of articles have indicated that the outwardly directed proton gradient stimulation of intestinal uptake of other cationic compounds occurs by a different mechanism. It has been documented that intestinal transport of tryptamine (Sugawara et al., 1992, 1995), enoxacin (Iseki et al., 1992), and disopyramide (Takahashi et al., 1993) might not be a function of an antiport system, but is, rather, due to electrophoretic mobility driven by an H\(^+\) diffusion potential.

In the presence of a proton gradient and permeant counterion, voltage clamping potential difference to zero produced a significant decrease in cimetidine uptake (Fig. 5). In addition, a further decrease was observed by dissipation of the proton gradient with FCCP. This was most notable at early time points, and is supportive of the possible involvement of a cation-proton exchange mechanism in intestinal cimetidine transport. A driving force contribution from both membrane potential and proton exchange could be the result of a membrane potential effect on an organic cation-H\(^+\) antiporter (Turner, 1981). Initial membrane binding followed by potential-driven translocation in parallel with a cimetidine-proton exchanger might provide an alternative accounting for the data. In a recent study of cimetidine uptake into syncytial microvillus membrane vesicles from human term placenta, FCCP data under voltage-clamped conditions suggested the involvement of an exchange mechanism in parallel with other pathways (Van der Aa et al., 1996). The low affinity of saturable transport in these placental vesicles (K\(_m\) = 6.3 mM) is similar to that determined in the intestinal vesicles used in this study.

In the case of kidney and choroid plexus brush-border vesicles, acceleration of cimetidine uptake in the presence of an outwardly directed proton gradient is consistent with cimetidine secretion from these organ systems. Under physiologic conditions, intracellular pH in the enterocytes is close to neutral (≈6.90). The pH in the mucosal microclimate of the brush-border membrane of jejunal enterocytes is known to be significantly acidic compared with the pH of the luminal fluid (Lucas, 1984); this is primarily mediated through a brush-border Na\(^+\)-H\(^+\) exchanger (Iwatsubo et al., 1986). Thus, there exists an H\(^+\) gradient across the intestinal brush-border membrane in the lumen-to-cell direction. The outwardly driven H\(^+\) gradient stimulation of cimetidine uptake uncovered in this study might signify a transport mechanism to drive secretion of cimetidine to the intestinal lumen as has been suggested for the organic cation, guanidine (Miyamoto et al., 1988). In fact, a 5-fold higher cimetidine flux in the serosal-to-mucosal direction as compared to the mucosal-to-serosal direction has been recently reported across intestinal Caco-2 monolayers (Pade and Stavchansky, 1997). The efflux experiment performed in this study, in a single preparation with cimetidine-loaded rat jejunal BBMV, is supportive of a proton-driven secretion consistent with microclimate pH conditions in rat jejunum.

Recently, a study on cimetidine secretion in cultured renal epithelial cell monolayers reports that net secretion across the apical brush-border membrane is a function of P glycoprotein-mediated secretion countered by cimetidine absorption via a proton-coupled diisothiocyanostilbene-2,2'-disulfonic acid-sensitive transport mechanism (Dudley and Brown, 1996). It has been shown in other systems that drug efflux mediated by P glycoprotein was not affected by changes in intracellular pH (Goda et al., 1996). The appearance of cimetidine sulfoxide in rat jejunal lumen from perfusion of cimetidine was inhibited by diisothiocyanostilbene-2,2'-disulfonic
acid (Hui et al., 1994), which could result from inhibition of cimetidine mucosal transport (Table 1).

Concentration dependence and inhibition experiments were carried out under proton gradient conditions that favored cimetidine accumulation in the vesicles. Because high cimetidine binding to vesicle membranes complicated interpretation of uptake studies, uptake of cimetidine into rat intestinal BBMV was obtained by subtracting the uptake of cimetidine from that measured in vesicle-fractured preparations. The uptake of cimetidine by rat intestinal BBMV exhibited nonlinear kinetics over the concentration range from 25 μM to 40 mM (Fig. 7). Cimetidine uptake into rat BBMV was saturable and, using nonlinear regression analysis of the uptake versus concentration curve, it was estimated that 91% of cimetidine uptake, over an initial 30-s interval, represented a saturable component. Furthermore, binding-corrected cimetidine uptake into BBMV was temperature-dependent.

Cimetidine initial (30-s) uptake showed self-inhibition and was inhibited by a related H₂-antagonist, ranitidine (Table 1). However, the similar levels of inhibition observed at equilibrium (4 h) uptake suggests that initial self-uptake inhibition could be entirely attributable to binding. Cimetidine uptake was inhibited by various organic cations but not by TEA or the organic anion, probenecid (Table 1), both of which inhibit cimetidine uptake in rabbit kidney luminal membranes (Gisclon et al., 1987). Lack of TEA inhibition is similar to observations of cimetidine transport across choroid plexus brush-border (Whittico et al., 1990). However, initial cimetidine uptake in bovine choroid plexus vesicles was inhibited to 28% of control uptake by histidine. Histidine inhibition was not observed for initial uptake of cimetidine in rat jejunal vesicles (Table 1).

Lipophilic weak bases including imipramine and erythromycin extensively inhibited cimetidine uptake; imipramine was the most potent inhibitor among the compounds tested. It has been reported that imipramine inhibited equilibrium guanidine uptake in rabbit BBMV by interacting with a carrier (Miyamoto et al., 1988). However, changes in surface potential in the presence of organic amines such as imipramine and tetracaine have been reported by Sugawara et al. (1995).

The possibility that intestinal cimetidine transport may be mediated by an intestinal carrier for cystoneine is of special interest as this agent is given orally to treat cytostisnosis. A cystoneine carrier has recently been characterized in lysosomal membranes (Pisoni et al., 1995) and cimetidine transport by a cystoneine carrier in the small intestinal brush-border membrane would represent a novel finding. Failure of cystoneine to inhibit initial cimetidine uptake may be consistent with the fact that it is not a substrate for the cystoneine lysosomal carrier. Greater inhibition at equilibrium than initial uptake for cystoneine and cymeine is a puzzling result. The fact that 2-(ethylthio)ethylamine inhibited initial but not equilibrium uptake may indicate the existence of a transport mechanism for an endogenous aminothioether related to enterocyte thiol biochemistry.

Based on this data for cimetidine uptake in rat jejunal BBMV, it might have been expected that cimetidine transport occurs via the guanidine-H⁺ antiporter. Of special interest in this regard is a recent report that both cimetidine and guanidine increased the intestinal permeation of the anions cefazolin and phenol red by inhibiting their intestinal secretion across the mucosal membrane of rat intestine (Saitoh and Aungst, 1995). However, cimetidine did not compete with guanidine uptake in the rabbit small intestinal BBMV study, indicating that cimetidine was not a substrate for the intestinal organic cation-H⁺ antiport system reported by Miyamoto et al. (1988). Unpublished studies by A. Tsuji et al. also showed that guanidine did not inhibit cimetidine uptake by rat intestinal brush-border membranes, whereas H₂-antagonists did inhibit cimetidine uptake.

In conclusion, a saturable component for cimetidine transport in rat intestinal brush-border membrane was characterized with respect to transport driving force and inhibition profile. Concentration and temperature dependence of binding-corrected uptake and a unique inhibition profile point toward a carrier-mediated uptake mechanism. The driving force data indicates that jejunal cimetidine transport may include both cation-H⁺ antiport and a potential driven uptake after an initial membrane-binding step.

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References


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