*Pharmaceutical Research, Vol. 23, No. 6, June 2006 (© 2006)*
DOI: 10.1007/s11095-006-0251-4

*Research Paper*

# Saturable Absorptive Transport of the Hydrophilic Organic Cation Ranitidine in Caco-2 Cells: Role of pH-Dependent Organic Cation Uptake System and P-Glycoprotein

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*Received July 15, 2005; accepted February 14, 2006*

***Purpose.*** The purpose of this work was to investigate the involvement of carrier-mediated apical (AP) uptake and efflux mechanisms in the absorptive intestinal transport of the hydrophilic cationic drug ranitidine in Caco-2 cells.

***Methods.*** Absorptive transport and AP uptake of ranitidine were determined in Caco-2 cells as a function of concentration. Permeability of ranitidine in the absorptive and secretory directions was assessed in the absence or presence of the P-glycoprotein (P-gp) inhibitor, GW918. Characterization of the uptake mechanism was performed with respect to inhibitor specificity, pH, energy, membrane potential, and $Na^+$ dependence. Efflux from preloaded monolayers was evaluated over a range of concentrations and in the absence or presence of high extracellular ranitidine concentrations.

***Results.*** Saturable absorptive transport and AP uptake of ranitidine were observed with $K_m$ values of 0.27 and 0.45 mM, respectively. The ranitidine absorptive permeability increased and secretory permeability decreased upon inhibition of P-gp. AP ranitidine uptake was inhibited in a concentration-dependent fashion by a diverse set of organic cations including tetraethylammonium, 1-methyl-4-phenylpyridinium, famotidine, and quinidine. AP ranitidine uptake was pH and membrane potential dependent and reduced under conditions that deplete metabolic energy. Efflux of [$^3$H]ranitidine across the basolateral membrane was neither saturable as a function of concentration nor *trans* stimulated by unlabeled ranitidine.

***Conclusions.*** Saturable absorptive transport of ranitidine in Caco-2 cells is partially mediated via a pH-dependent uptake transporter for organic cations and is subject to attenuation by P-gp. Inhibition and driving force studies suggest the uptake carrier exhibits similar properties to cloned human organic cation transporters. The results also imply ranitidine transport is not solely restricted to the paracellular space.

**KEY WORDS:** carrier-mediated transport; intestinal absorption; organic cation transport; P-glycoprotein; ranitidine.

## INTRODUCTION

$H_2$ receptor antagonists have been successfully used in clinical practice for the treatment of ulcers and reduction of gastric acid secretion in humans. As a class, the $H_2$ receptor antagonists including ranitidine, famotidine, and cimetidine exhibit similar properties with respect to their pharmacokinetic behavior. In general, they are relatively well absorbed from the gastrointestinal tract ($F$ ~40–70%), not extensively metabolized, and are primarily eliminated unchanged via transporter-assisted renal tubular secretion in the kidney (1). The favorable absorption properties of these compounds, however, are not entirely consistent with their physical and chemical properties. $H_2$ receptor antagonists are weakly basic, hydrophilic compounds that exist as partially charged organic cations at intestinal pH values (e.g., ranitidine, $pK_a$ 8.2). Their relatively low lipophilicity [e.g., ranitidine, log $P$ 0.27 (2)], large number of hydrogen bond donor and acceptor sites and net positive charge at physiological pH values would suggest poor passive membrane permeability. This dichotomy between such poor physicochemical properties and their relatively efficient absorption *in vivo* suggests a potential role for intestinal transporters in the oral absorption of these and other hydrophilic cations that remains to be fully elucidated.

Previous investigations utilizing Caco-2 cell monolayers, a well-characterized intestinal epithelial cell culture model,

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**ABBREVIATIONS:** AP, apical; BL, basolateral; $CL_{eff}$, efflux clearance; HBSS, Hank's balanced salt solution; $MPP^+$, 1-methyl-4-phenyl pyridinium; NMDG, *N*-methyl-D-glucamine; OCT, organic cation transporter; OCTN, novel organic cation transporter; P-gp, P-glycoprotein; TEA, tetraethylammonium; TMA, tetramethylammonium; 2,4-DNP, 2,4-dinitrophenol.

have suggested that the absorptive (mucosal to serosal) transport of ranitidine occurs predominantly via the paracellular route (3,4). Such conclusions were based primarily on the significant increase in permeability of ranitidine observed under conditions known to compromise the integrity of the tight junctions (e.g., $Ca^{2+}$ depletion) (3). Interestingly, the absorptive transport and permeability of ranitidine and famotidine across Caco-2 cell monolayers also was found to be saturable as a function of concentration suggesting a mechanism capable of facilitating their transport across the monolayers (5). A novel mechanism involving saturable transport within the paracellular space mediated by charge–charge interaction between the cationic moiety of ranitidine (and famotidine) and anionic residues within the paracellular space and/or tight junction was proposed (5). Such a mechanism was consistent with the postulated predominantly paracellular mechanism of ranitidine absorptive transport, explained the increase observed in the transepithelial electrical resistance (TEER) across the monolayers in the presence of such compounds, and was consistent with the inhibition of ranitidine transport by known inhibitors of the paracellular ionic conductance (e.g., 2,4,6-triaminopyrimidine) (5,6).

Although the previous data were consistent with a saturable paracellular mechanism, the potential for carrier-mediated mechanisms to play a role in the absorptive transport of such compounds in Caco-2 cells could not be ruled out. Recent studies have identified various $H_2$ receptor antagonists as substrates for organic cation (7–9) and organic anion (10,11) transporters, and carrier-mediated mechanisms have been identified in their transport in a number of tissues including the choroid plexus (11), liver (12), and kidney (13,14). Furthermore, the $H_2$ receptor antagonist cimetidine exhibits saturable uptake into brush-border membrane vesicles isolated from rat small intestine suggesting carrier-mediated transport mechanisms also may be present in the intestine (15). Despite this initial report, however, the role of carrier-mediated transport in the intestinal absorption of $H_2$ receptor antagonists is not clear.

Investigation into the secretory (serosal to mucosal) transport of ranitidine and cimetidine has surprisingly implicated a role for the efflux transporter, P-glycoprotein (P-gp), in the intestinal transport of these hydrophilic cations. Collett *et al.* initially reported a reduced secretory transport of cimetidine and ranitidine in the presence of P-gp inhibitors in both Caco-2 cells and rat intestine suggesting a role for P-gp in their secretory transport (4). These results were later confirmed in the Caco-2 model and extended to include famotidine (16). Interestingly, the effect of P-gp on ranitidine and cimetidine transport was asymmetric such that the secretory transport was affected to a much greater extent than the absorptive transport in the presence of the nonspecific P-gp inhibitor verapamil (4). Experimental evidence suggests that such an asymmetric effect likely results from differing absorptive and secretory transport mechanisms and/or differing apparent affinity (e.g., apparent $K_m$) of substrates for P-gp in the absorptive *vs.* secretory directions as a result of differences in passive permeability across the apical (AP) and basolateral (BL) membranes or the involvement of multiple transporters (17,18). In light of the potential involvement of carrier-mediated and paracellular mechanisms in the absorptive transport of ranitidine, the role of P-gp-mediated efflux in the absorptive transport of such compounds requires further examination.

The purpose of the current study was to examine the potential involvement of a saturable, carrier-mediated uptake process in the absorptive transport of the model hydrophilic organic cation ranitidine in the Caco-2 model of intestinal epithelium. The results clearly suggest that saturable carrier-mediated uptake of ranitidine in Caco-2 cells contributes to its overall absorptive transport. Furthermore, AP uptake and accumulation of ranitidine could be modulated by both typical organic cation transporter (OCT) substrates/inhibitors and a potent P-gp inhibitor suggesting a role for both uptake and efflux transport proteins in the net cellular accumulation of ranitidine across the AP membrane of Caco-2 cells. Finally, the BL efflux of ranitidine following its intracellular accumulation was examined to evaluate the rate-limiting step in the carrier-mediated transcellular pathway for absorptive transport of ranitidine across Caco-2 cells.

## MATERIALS AND METHODS

### Materials

The Caco-2 cell line, Caco-2 cell clone P27.7 (19), was obtained from Mary F. Paine, Ph.D., and Paul B. Watkins, M.D. (Schools of Pharmacy and Medicine, The University of North Carolina at Chapel Hill, Chapel Hill, NC, USA). Eagle's minimum essential medium (EMEM) with Earle's salts and L-glutamate, nonessential amino acids (NEAA, 100×), 0.05% trypsin–0.53 mM EDTA solution, and penicillin–streptomycin–amphotericin B solution (100×) were obtained from Gibco Laboratories (Grand Island, NY, USA). Fetal bovine serum (FBS) was obtained from Sigma Chemical Co. (St. Louis, MO, USA). Hank's balanced salt solution (HBSS) was obtained from Mediatech, Inc. (Herndon, VA, USA). HEPES (1 M) was obtained from Lineberger Comprehensive Cancer Center at the University of North Carolina at Chapel Hill. 2-[*N*-Morpholino]ethanesulfonic acid (MES), *N*-methyl-D-glucamine (NMDG), 2,4-dinitrophenol (2,4-DNP), tetraethylammonium (TEA) chloride, 1-methyl-4-phenyl pyridinium ($MPP^+$), quinidine, L-carnitine hydrochloride, L-phenylalanine, glycyl–sarcosine (Gly–Sar), probenecid, taurocholic acid, and benzoic acid were purchased from Sigma. Tetramethylammonium (TMA) chloride was purchased from Fisher Scientific (Pittsburgh, PA, USA). Famotidine was purchased from ICN Biomedicals (Aurora, OH, USA). Unlabeled ranitidine was purchased from Research Biochemicals International (Natick, MA, USA). [$^3$H]Gly–Sar (30 Ci/mmol) and [$^3$H]$MPP^+$ (85 Ci/mmol) were obtained from American Radiolabeled Chemicals (St. Louis, MO, USA). [$^3$H]Ranitidine (7 Ci/mmol) was obtained as a gift from GlaxoSmithKline (Research Triangle Park, NC, USA) and originally custom synthesized by Amersham Life Sciences (Piscataway, NJ, USA). [$^3$H]Ranitidine was purified immediately prior to experiments by reverse-phase high-performance liquid chromatography (HPLC) and radiochemical purity exceeded 90% as verified by radio-HPLC detection (Flow Scintillation Analyzer 500TR Series, Packard Bio-

science, Downer's Grove, IL, USA). GW918 (20) was obtained as a gift from GlaxoSmithKline.

## Cell Culture

Caco-2 cells were cultured at 37°C in EMEM, supplemented with 10% FBS, 1% NEAA, 100 U/mL penicillin, 100 μg/mL streptomycin, and 0.25 μg/mL amphotericin B in an atmosphere of 5% $CO_2$ and 90% relative humidity. The cells were passaged upon reaching 90% confluency using trypsin–EDTA, and plated at densities of 1:5 in 75-$cm^2$ T flasks. Caco-2 cells (passage number 50 to 65) were seeded at a density of 60,000 cells/$cm^2$ on polycarbonate membranes of Transwells™ (12 mm i.d., 0.4 μm pore size, Costar, Cambridge, MA, USA). Medium was changed the day after seeding and every other day thereafter (AP volume 0.5 mL, BL volume 1.5 mL). The Caco-2 cell monolayers were used 20–25 days postseeding. TEER was measured to ensure cell monolayer integrity. Measurements were obtained using an EVOM Epithelial Tissue Voltammeter and an Endohm-12 electrode (World Precision Instruments, Sarasota, FL, USA). Cell monolayers with TEER values greater than 300 Ω $cm^2$ were used in transport experiments.

## Transport Studies

Transport studies were conducted as described previously with minor deviations (5). Cell monolayers were preincubated for 30 min at 37°C in transport buffer solution (HBSS with 25 mM D-glucose and 10 mM HEPES, pH 7.2). Transport experiments were initiated by replacing the donor solution (AP for absorptive transport, BL for secretory transport) with 0.4 mL (AP) or 1.5 mL (BL) of transport buffer containing various ranitidine concentrations or ranitidine in the presence of various inhibitors. The pH in both AP and BL compartments was 7.2 for all transport experiments. Appearance of ranitidine in the receiver compartment (BL for absorptive transport, AP for secretory transport) was evaluated as a function of time in the linear region of transport and under sink conditions. For transport experiments investigating the role of P-gp, cell monolayers were preincubated in the absence or presence of GW918 (1 μM) in both AP and BL compartments for 30 min. Ranitidine transport was evaluated in the absence or presence of GW918 (1 μM) added to both donor and receiver compartments. At the conclusion of the experiment, accumulation of ranitidine in the cells was determined after washing three times with 4°C transport buffer. Monolayers were allowed to dry, excised from the Transwell™, and dissolved in 300 μL 1% Triton X-100 for 4 h with shaking. The solution was centrifuged at 10,000 rpm for 10 min and the supernatant was analyzed by HPLC-UV (Agilent Technologies 1100 Series, Palo Alto, CA, USA). Protein content was determined by the BCA protein assay (Pierce, Rockford, IL, USA) with bovine serum albumin as a standard. TEER was measured at the beginning and end of each experiment to ensure monolayer integrity throughout the course of the experiment. Cell monolayers with final TEER ≤300 were discarded.

## Uptake Studies

### *Uptake Kinetics*

All uptake studies were conducted in Caco-2 cells grown on Transwell™ filters. Cell monolayers were preincubated for 30 min at 37°C in transport buffer in the absence or presence of GW918 (1 μM). Experiments were initiated by replacement of the AP transport buffer with 0.4 mL of transport buffer containing varying ranitidine concentrations in the absence or presence of GW918 (1 μM). The pH in both AP and BL compartments was 7.2 for uptake kinetics experiments. Uptake was determined over 2 min (within the linear uptake region) after which the AP solution was aspirated and monolayers washed three times with 4°C transport buffer. Monolayers were allowed to dry, excised from the Transwell™, and dissolved in 300 μL 1% Triton X-100 by incubating for 4 h with shaking. The solution was centrifuged at 10,000 rpm for 10 min and the supernatant analyzed by HPLC-UV (Agilent 1100 Series). Protein content was determined by the BCA protein assay (Pierce) with bovine serum albumin as a standard.

### *Inhibition Studies*

Cell monolayers were preincubated for 30 min at 37°C with transport buffer in the presence of GW918 (1 μM). The AP buffer was replaced with 0.4 mL of transport buffer containing ranitidine (0.1 mM) and GW918 (1 μM) in the absence or presence of various concentrations of inhibitors. The pH in both AP and BL compartments was 7.2 for all inhibition studies. Uptake was determined over 5 min after which the AP solution was aspirated and monolayers washed three times with 4°C transport buffer. Cell monolayers were assayed for ranitidine and protein content as described under "Uptake kinetics." $IC_{50}$ values for inhibition of ranitidine uptake were determined from the quinidine-sensitive portion of ranitidine uptake. Quinidine (200 μM) completely abolished the saturable portion of ranitidine (0.1 mM) uptake as determined in preliminary experiments. A sigmoidal inhibition model was used to determine $IC_{50}$ values as described under "Data analysis."

### *Driving Force Studies*

*Extracellular pH.* Cell monolayers were preincubated with transport buffer of various pH (6.0, 6.5, 7.2, 8.0) on the AP side and pH 7.2 on the BL side for 30 min at 37°C. Transport buffer was buffered with 10 mM MES (pH 6.0, 6.5) or 10 mM HEPES (pH 7.2, 8.0). The AP buffer was replaced with 0.4 mL of the appropriate pH transport buffer containing ranitidine (0.1 mM) in the absence or presence of quinidine (200 μM). Uptake was determined over 2 min after which the AP solution was aspirated and monolayers washed three times with 4°C transport buffer. Cell monolayers were assayed for ranitidine and protein content as described under "Uptake kinetics."

*Sodium Dependence.* Cell monolayers were preincubated with transport buffer in the absence or presence of $Na^+$ ions for 30 min at 37°C. NaCl (137 mM) was iso-

osmatically replaced with NMDG chloride for studies performed under $Na^+$-free conditions. Ranitidine (0.1 mM) uptake solutions also were prepared in the absence or presence of $Na^+$. The pH was 7.2 in both AP and BL compartments. Uptake was determined over 5 min after which the AP solution was aspirated and monolayers washed three times with 4°C transport buffer. Cell monolayers were assayed for ranitidine and protein content as described under "Uptake kinetics."

*Membrane Potential Dependence.* Cell monolayers were preincubated in normal transport buffer or a high-$K^+$ transport buffer in the presence of the potassium ionophore valinomycin (1 μM) for 30 min at 37°C. The high-$K^+$ buffer was prepared by replacing NaCl with KCl to achieve final KCl and NaCl concentrations of 137 and 5 mM, respectively. Ranitidine (0.1 mM) and [$^3$H]$MPP^+$ (1 μM; 1 μCi/mL) uptake solutions were also prepared under normal or high-$K^+$ buffer conditions. The pH in both AP and BL compartments was 7.2. Uptake was determined over 5 min after which the AP solution was aspirated and monolayers washed three times with 4°C transport buffer. Cell monolayers were assayed for ranitidine and protein content as described under "Uptake kinetics."

*Energy Dependence.* Cell monolayers were preincubated in the absence or presence of the metabolic energy inhibitor 2,4-DNP (1 mM) for 15 min at 37°C. Ranitidine (0.1 mM), [$^3$H]Gly–Sar (0.1 mM; 1 μCi/mL), and [$^3$H]$MPP^+$ (1 μM; 1 μCi/mL) uptake solutions were also prepared in the absence or presence of 2,4-DNP (1 mM). The pH of the AP solution for the Gly–Sar uptake experiment was adjusted to 6.0 (buffered with MES) because Gly–Sar is a substrate of the $H^+$-coupled dipeptide transporter. All other studies were conducted at a pH of 7.2 in both the AP and BL compartments. Uptake was determined over 5 min after which the AP solution was aspirated and monolayers washed three times with 4°C transport buffer. Cell monolayers were assayed for ranitidine and protein content as described under "Uptake kinetics."

### Efflux Studies

Cell monolayers were preincubated in transport buffer for 30 min at 37°C. Cells were then preloaded from the AP side only (to simulate conditions observed under AP to BL transport) for 30 min with 1, 100, or 1000 μM [$^3$H]ranitidine (0.75 μCi/mL). After loading, the uptake solution was aspirated and cell monolayers were washed three times with 4°C transport buffer. Prewarmed (37°C) transport buffer was added to both AP (0.4 mL) and BL (1.5 mL) sides and efflux of [$^3$H]ranitidine evaluated as a function of time by sampling (200 μL) from the AP and BL compartments. Removed aliquots were replaced with fresh transport buffer (37°C) after sampling. All solutions in both AP and BL compartments were at pH 7.2. [$^3$H]Ranitidine samples were analyzed by liquid scintillation counting (1600 TR Liquid Scintillation Analyzer, Packard Instrument Company, Downers Grove, IL, USA).

### HPLC Analysis

The amount of ranitidine transported or accumulated in the Caco-2 cells was quantified by HPLC (Agilent 1100 Series) using a 100 × 3 mm C18 Aquasil column (5 μM; Keystone Scientific, Inc. Bellefonte, PA, USA). Transport samples were analyzed using an isocratic mobile phase [12% acetonitrile (ACN) and 88% 50 mM phosphate buffer, pH 6.0] at a flow rate of 0.7 mL/min. Uptake samples were analyzed using a gradient method starting at (A) 12% ACN/(B) 88% 50 mM phosphate (pH 6.0) from 0 to 8 min. Composition increased linearly to 60% (A)/40% (B) from 8 to 12 min and was held constant at 60% (A)/40% (B) from 12 to 18 min. Composition reverted back to 12% (A)/88% (B) at 18 min and was held constant from 18 to 30 min for reequilibration. Flow rate was held constant at 0.7 mL/min throughout the method. Ranitidine was detected by UV at 320 nm. Retention time for ranitidine was 7.1 min.

### Data Analysis

Data are expressed as mean ± SD from three measurements unless otherwise noted. Statistical significance was evaluated using unpaired *t* tests or ANOVA followed by Tukey's test for multiple comparisons as appropriate. Kinetic constants ($J_{max}$, $K_m$, $K_d$) were obtained by fitting a model incorporating saturable and nonsaturable components to the ranitidine transport and uptake data at 37°C. The following model was utilized:

$$J = \frac{J_{max}C}{K_m + C} + K_d C \quad (1)$$

where $J_{max}$ is the maximal transport/uptake rate, $K_m$ is the kinetic constant for saturable transport/uptake, $K_d$ is the kinetic constant for nonsaturable transport/uptake, and $C$ is the ranitidine concentration. Apparent permeability ($P_{app}$) was determined using Eq. (2):

$$P_{app} = \frac{dQ/dt}{A \cdot C_o} \quad (2)$$

where $dQ/dt$ is the flux determined from the amount transported ($Q$) over time ($t$) during the experiment, $A$ is the surface area of the porous membrane, and $C_o$ is the initial concentration in the donor side. $IC_{50}$ values were determined by fitting a sigmoidal inhibition model to the inhibition data using Eq. (3):

$$V = \frac{V_o}{[1 + (I/IC_{50})^n]} \quad (3)$$

where $V$ is the uptake rate of ranitidine in the presence of inhibitor, $V_o$ is the uptake rate of ranitidine in the absence of inhibitor, $I$ is the concentration of inhibitor, and $n$ is the Hill coefficient. WinNonlin (Pharsight, Mountain View, CA, USA) was used for estimation of kinetic constants and $IC_{50}$ values. Efflux clearance ($CL_{eff}$) was calculated using Eq. (4):

$$CL_{eff} = \frac{dX/dt}{C_o} \quad (4)$$

where $dX/dt$ is the amount effluxed ($X$) over time ($t$) determined in the linear region of efflux and $C_o$ is the initial concentration of ranitidine loaded in the cells. Initial ranitidine intracellular concentrations were calculated using the amount loaded at $t = 0$ and a Caco-2 cellular volume of 3.66 μL/mg protein (21,22).

## RESULTS

### Concentration-Dependence of Ranitidine Absorptive (AP to BL) Transport and AP Uptake in Caco-2 Cell Monolayers

The absorptive transport and AP uptake of ranitidine were evaluated as a function of concentration. Ranitidine absorptive transport was concentration dependent and the data were well described by a model consisting of one saturable and one nonsaturable term as previously observed (5) (Fig. 1A). The $J_{max}$ and $K_m$ estimated for absorptive ranitidine transport were 65.0 ± 3.6 pmol $min^{-1}$ $cm^{-2}$ and 0.27 ± 0.04 mM, and compared favorably to the values previously reported ($J_{max}$, 45.6 ± 5.5; $K_m$, 0.48 ± 0.11) (5). The estimated coefficient of nonsaturable transport, $K_d$, was 22.0 ± 0.80 nL $min^{-1}$ $cm^{-2}$. Comparison of $K_d$ with the saturable transport clearance ($J_{max}/K_m$, 241 nL $min^{-1}$ $cm^{-2}$) suggests that ~90% of the overall ranitidine transport at low concentrations ($\ll K_m$) results via a saturable process. The cellular uptake of ranitidine (0.5 mM) across the AP membrane was rapid and reached steady state by 10 min (Fig. 1B, inset) suggesting that ranitidine is accumulated across the AP membrane in Caco-2 cells. The initial AP uptake rate (approximated at 2 min) of ranitidine as a function of concentration at 37°C was saturable and also well described by a model consisting of one saturable and one nonsaturable term (Fig. 1B). The $J_{max}$ and $K_m$ estimated for AP ranitidine uptake were 680 ± 79 pmol $min^{-1}$ mg $protein^{-1}$ and 0.45 ± 0.09 mM, respectively. The nonsaturable component of uptake, $K_d$, was estimated to be 0.264 ± 0.020 μL $min^{-1}$ mg $protein^{-1}$. Comparison of $K_d$ with the saturable uptake clearance ($J_{max}/K_m$: 1.51 μL $min^{-1}$ mg $protein^{-1}$) suggests that ~85% of the overall ranitidine uptake at low concentrations ($\ll K_m$) results via a saturable process. The uptake of ranitidine at 4°C also was linear as a function of concentration and significantly lower than the uptake at 37°C (Fig. 1B). The data are consistent with a saturable, carrier-mediated AP uptake process for ranitidine in Caco-2 cells.

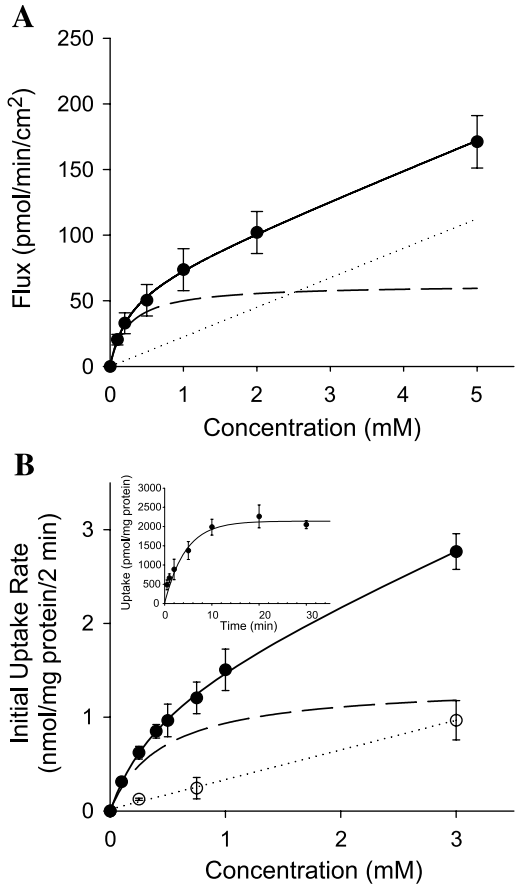


**Fig. 1.** Concentration dependence of the absorptive transport and AP uptake of ranitidine in Caco-2 cells. (A) AP to BL flux of ranitidine (●) was determined at the indicated concentrations across Caco-2 cell monolayers. The solid, dashed, and dotted lines represent the best fit to the overall transport data, saturable component of transport, and nonsaturable component of transport, respectively. (B) AP uptake of ranitidine in Caco-2 cells was determined over 2 min at the indicated concentrations at either 37°C (●) or 4°C (○). The solid and dashed lines represent the best fit of the 37°C ranitidine uptake data and the saturable component of ranitidine uptake, respectively. The dotted line represents linear regression of the 4°C uptake data. The inset depicts the accumulation of ranitidine (0.5 mM) in Caco-2 cells as a function of time. All experiments were conducted at an AP and BL pH of 7.2. Data represent mean ± SD; $n$=3.

### Role of P-gp on Ranitidine Transport and Accumulation in Caco-2 Cells

The role of P-gp upon ranitidine absorptive and secretory transport as well as intracellular accumulation was assessed by examining these processes in the absence or presence of the potent P-gp inhibitor, GW918 (1 μM). Secretory permeability ($P_{app,BA}$) of ranitidine (0.1 mM) was greater than absorptive permeability ($P_{app,AB}$) under control conditions indicating apically directed secretion of ranitidine ($P_{app,BA}/P_{app,AB}$=1.6) (Fig. 2A). In the presence of GW918 (1 μM), $P_{app,AB}$ significantly increased and $P_{app,BA}$ significantly decreased consistent with inhibition of P-gp ($p$<0.05) (Fig. 2A). Interestingly, the efflux ratio ($P_{app,BA}/P_{app,AB}$) in the presence of GW918 did not collapse to ~1, but was reduced to a value <1, thus unmasking a significant polarity in the absorptive transport direction ($P_{app,BA}/P_{app,AB}$=0.69) in the absence of P-gp-mediated efflux of ranitidine. No difference was observed in the permeability of mannitol in the presence of GW918 (1 μM) indicating that GW918 does not affect paracellular transport (data not shown). As expected, the cellular accumulation of ranitidine (0.1 mM) from either the AP or BL side of the monolayers increased significantly in the presence of GW918 (1 μM) ($p$<0.05) (Fig. 2B). The results suggest a small but significant role for P-gp in the transport of ranitidine and imply the presence of a transcellular component to ranitidine absorptive transport.

### *Cis* Inhibition of Ranitidine AP Uptake and Absorptive Transport by Organic Cations in Caco-2 Cells

Saturable AP uptake of ranitidine was further characterized by competition studies with potential inhibitors of carrier-mediated uptake systems. Preliminary studies indicated that P-gp slightly attenuated (although not statistically significant) AP uptake of ranitidine at the initial time point

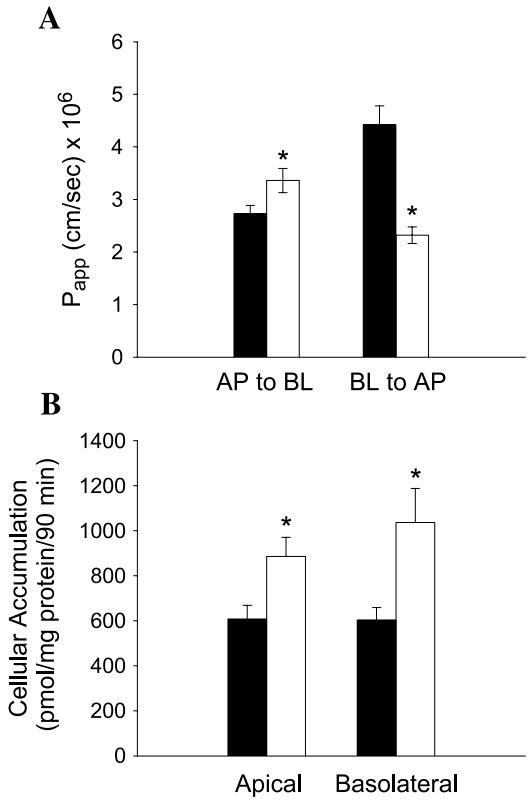


**Fig. 2.** Effect of P-gp inhibitor GW918 on ranitidine transport across and accumulation into Caco-2 cell monolayers. (A) Permeability of ranitidine (0.1 mM) was determined in the absorptive (AP to BL) and secretory (BL to AP) directions in the absence (solid bars) or presence (open bars) of GW918 (1 μM). (B) Cellular accumulation of ranitidine (0.1 mM) at the conclusion of the transport study after AP or BL dosing in the absence (solid bars) or presence (open bars) of GW918 (1 μM). All experiments were conducted at an AP and BL pH of 7.2. Data represent mean ± SD; $n$=3. *$p$<0.05.

(5 min) used for inhibition studies (data not shown). All inhibition studies were thus conducted in the presence of GW918 (1 μM) to eliminate any potential effect of P-gp-mediated efflux on the uptake data. The expression and function of AP uptake transporters such as hPepT1, LNAA, MCT, and IBAT in Caco-2 cells has been well documented (23). Prototypical substrates of each of these transporters [hPepT1: Gly–Sar (5 mM); LNAA: L-phenylalanine (1 mM); MCT: benzoic acid (10 mM); IBAT: taurocholate (0.5 mM)] did not significantly affect AP uptake of ranitidine (0.1 mM) in Caco-2 cells suggesting none are involved in saturable ranitidine uptake (data not shown). Significant inhibition of ranitidine (0.1 mM) AP uptake was observed in the presence of various organic cations including TEA (5 mM), $MPP^+$ (0.5 mM), quinidine (200 μM), and famotidine (5 mM) ($p$<0.05) (Fig. 3A). The organic cation TMA (5 mM), zwitterion L-carnitine (1 mM), and organic anion transport inhibitor probenecid (2 mM) did not significantly affect AP ranitidine uptake (Fig. 3A). The saturable portion of ranitidine transport at 0.1 mM was calculated using Eq. (1) and the kinetic parameters associated with AP ranitidine uptake, and was estimated to account for ~80% of the total uptake. Therefore, quinidine (200 μM) nearly completely abolished saturable AP uptake of ranitidine (~75% inhibition). Importantly, quinidine (200 μM) exhibited no effect upon ranitidine (0.1 mM) uptake at 4°C indicating inhibition was primarily directed against intracellular uptake as opposed to potential membrane binding (see Fig. 3 legend).

Significant inhibition of ranitidine (0.1 mM) absorptive transport (Fig. 3B) also was observed in the presence of TEA (5 mM), $MPP^+$ (0.5 mM), quinidine (200 μM), and famotidine (5 mM) ($p$<0.05). A similar magnitude of inhibition was observed for initial AP uptake (Fig. 3A) and absorptive transport (Fig. 3B). TMA (5 mM), L-carnitine (1 mM), and probenecid (2 mM) exhibited minimal inhibition of both absorptive transport and AP uptake (Fig. 3).

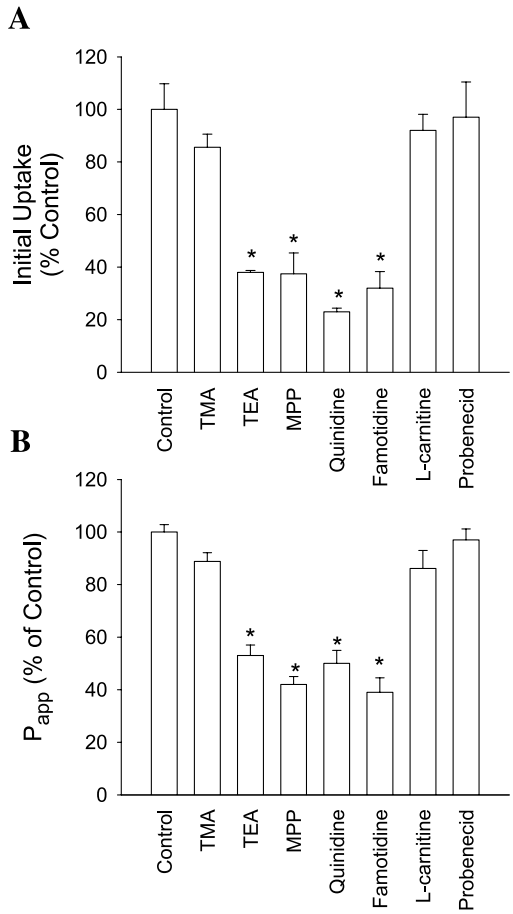


**Fig. 3.** *Cis* inhibition of the AP uptake (A) and absorptive transport (B) of ranitidine by various organic cations in Caco-2 cells. (A) Ranitidine (0.1 mM) uptake was determined in the absence or presence of TMA (5 mM), TEA (5 mM), $MPP^+$ (0.5 mM), quinidine (200 μM), famotidine (5 mM), L-carnitine (1 mM), and probenecid (2 mM) for 5 min at 37°C. GW918 (1 μM) was present in the uptake buffer to abolish P-gp-mediated efflux activity. Control uptake was 466.4 ± 44.2 pmol/mg protein per 5 min. Ranitidine uptake at 4°C was unaffected by the presence of quinidine [82 ± 21 (control) *vs.* 90 ± 25 (quinidine) pmol/mg protein per 5 min]. (B) Absorptive transport of ranitidine (0.1 mM) was determined in the absence or presence of TMA (5 mM), TEA (5 mM), $MPP^+$ (0.5 mM), quinidine (200 μM), famotidine (5 mM), L-carnitine (1 mM), and probenecid (2 mM). Control $P_{app}$ was (3.80 ± 0.11) × $10^{-6}$ cm/s. All experiments were conducted at an AP and BL pH of 7.2. Data represent mean ± SD; $n$=3. *$p$<0.05 compared to control.

### Potency of AP Ranitidine Uptake Inhibition by Selected Organic Cations

Potency of inhibition was assessed by evaluating ranitidine AP uptake in the presence of increasing concentrations of selected organic cations. Quinidine, TEA, $MPP^+$, and famotidine significantly inhibited ranitidine AP uptake in a concentration-dependent fashion (Fig. 4). Because quinidine virtually abolishes the saturable component of ranitidine uptake, the quinidine-sensitive (200 μM) portion of ranitidine uptake was used for determination of $IC_{50}$ values (Fig. 4). The $IC_{50}$ values determined for inhibition of ranitidine uptake revealed marked differences in the potencies of the examined organic cations to inhibit AP uptake of ranitidine. Quinidine exhibited the most potent inhibition ($IC_{50}$, 9.7 ± 1.0 μM), whereas inhibition by TEA was relatively weak ($IC_{50}$, 697 ± 213 μM). $MPP^+$ and famotidine inhibited ranitidine uptake with $IC_{50}$ values of 26.1 ± 6.9 and 148 ± 52 μM, respectively. The wide range of potencies suggests a specific transport process is involved in the AP uptake of ranitidine in Caco-2 cells and that selected inhibitors bind to the carrier with varying affinities.

### Effect of Extracellular pH on AP Ranitidine Uptake in Caco-2 Cells

The role of extracellular pH upon AP ranitidine uptake was evaluated over a pH range from 6.0 to 8.0. The total uptake of ranitidine (0.1 mM) significantly decreased as the extracellular pH was lowered (Fig. 5A). To determine the effect of pH on carrier-mediated uptake *vs.* passive uptake, ranitidine (0.1 mM) uptake across the AP membrane was evaluated in the absence or presence of quinidine (200 μM). Quinidine-sensitive ranitidine uptake approximates the saturable portion of uptake, whereas the quinidine-insensitive portion of uptake reflects the nonsaturable component. Both quinidine-sensitive (saturable) and quinidine-insensitive (nonsaturable) uptake decreased as the extracellular pH was lowered (Fig. 5B). The decrease in nonsaturable uptake presumably reflects decreased passive diffusion of the unionized form of ranitidine as a result of increased ionization (ranitidine, $pK_a$ 8.2). The decrease in saturable uptake could have resulted from a decrease in the driving force for an organic cation/$H^+$ exchange mechanism as has been observed for uptake of diphenhydramine in Caco-2 cells (24). However, intracellular acidification by $NH_4Cl$ prepulse (24–26) of Caco-2 cells failed to stimulate ranitidine AP uptake (data not shown) suggesting $H^+$ exchange is not the driving force behind ranitidine AP uptake and that a pH-sensitive transporter may be involved in the uptake process.

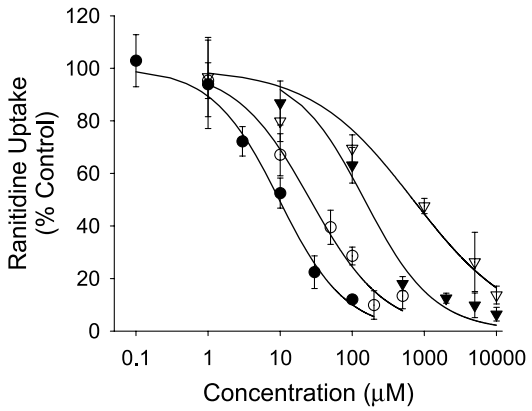


**Fig. 4.** Concentration-dependent inhibition of ranitidine AP uptake by various organic cations in Caco-2 cells. Uptake of ranitidine (0.1 mM) was determined in the absence or presence of increasing concentrations of quinidine (●), $MPP^+$ (○), famotidine (▼), and TEA (▽) for 5 min. GW918 (1 μM) was present in the uptake buffer to abolish P-gp-mediated efflux activity. Data represent inhibition of the quinidine-sensitive (200 μM) portion of ranitidine uptake. All experiments were conducted at an AP and BL pH of 7.2. Data represent mean ± SD; $n$=3.

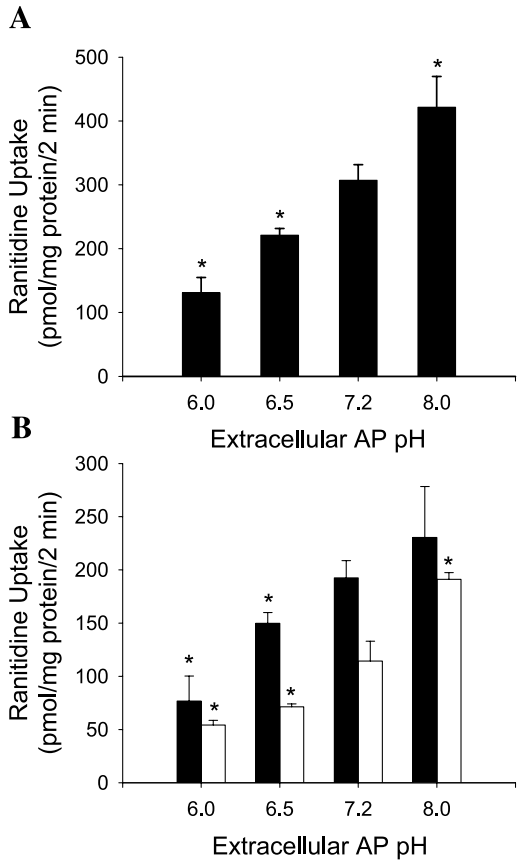


**Fig. 5.** pH-dependent AP ranitidine uptake in Caco-2 cells. (A) Total ranitidine (0.1 mM) uptake was determined at the indicated AP pH. pH of the BL compartment was 7.2. (B) Ranitidine (0.1 mM) uptake was determined in the absence or presence of quinidine (200 μM) at the indicated AP pH. pH of the BL compartment was 7.2. The quinidine-sensitive portion of ranitidine uptake (solid bars) was determined by subtracting ranitidine uptake in the presence of quinidine (200 μM) from the total ranitidine uptake. The quinidine-insensitive (passive) portion of ranitidine uptake (open bars) reflects the uptake of ranitidine in the presence of quinidine (200 μM). Data represent mean ± SD; $n$=3. *$p$<0.05 compared to the uptake determined at pH 7.2.

### Effect of Metabolic Energy Inhibition, $Na^+$ Dependence, and Membrane Potential on AP Ranitidine Uptake in Caco-2 Cells

The driving force for ranitidine AP uptake in Caco-2 cells was investigated via metabolic energy depletion, remov-

**Table I.** Effect of Metabolic Energy, $Na^+$ –Dependence, and Membrane Potential on AP Uptake of Ranitidine in Caco-2 Cells

| | Metabolic energy | | $Na^+$ dependence | | Membrane potential | |
|---|---|---|---|---|---|---|
| | Control (% of control) | 2,4-DNP (% of control) | Control (% of control) | $Na^+$ Free (% of control) | Control (% of control) | Depolarized (% of control) |
| Ranitidine (100 μM) | 100 (10) | 74 (7)* | 100 (13) | 90 (12) | 100 (6.0) | 88 (2.1)* |
| Gly–Sar[a] (100 μM) | 100 (3.2) | 60 (12)* | N.D. | N.D. | N.D. | N.D. |
| $MPP^+$ (1 μM) | 100 (19) | 84 (13) | N.D. | N.D. | 100 (18) | 59 (22)* |

Data represent mean (SD); $n$=3. N.D., not determined.
[a] Gly–Sar uptake conducted at AP pH 6.0. All other studies conducted at AP and BL pH of 7.2.
*$p$<0.05 compared to control.

al of extracellular $Na^+$ ions from the uptake buffer, and under conditions designed to abolish the inside negative membrane potential. Uptake of ranitidine (0.1 mM) and of the positive control, [$^3$H]Gly–Sar (0.1 mM), was significantly reduced in the presence of metabolic energy inhibitor 2,4-DNP (1 mM) suggesting a role for active transport processes in their uptake ($p$<0.05) (Table I). When sodium was removed from the uptake buffer and replaced with NMDG, the uptake of ranitidine (0.1 mM) was not significantly affected indicating that AP uptake of ranitidine is not driven by the inward sodium gradient (Table I). Depolarization of the membrane potential was induced by incubation of Caco-2 cells in a high-$K^+$ buffer in the presence of the $K^+$ ionophore, valinomycin (1 μM), for 30 min. Under depolarized conditions, uptake of ranitidine (0.1 mM) exhibited a small but statistically significant decrease in uptake compared to control ($p$<0.05) (Table I). Uptake of [$^3$H]$MPP^+$ in Caco-2 cells has previously been shown to be membrane potential dependent (27). As expected, AP uptake of [$^3$H]$MPP^+$ (1 μM) also was significantly decreased under depolarized conditions ($p$<0.05) (Table I). Thus, ranitidine AP uptake in Caco-2 cells is $Na^+$ independent and partially driven by an inside negative membrane potential.

### Efflux of [$^3$H]Ranitidine from Preloaded Caco-2 Cell Monolayers

Efficient vectorial transport across cell monolayers involves uptake across the AP membrane followed by subsequent efflux across the BL membrane. To investigate

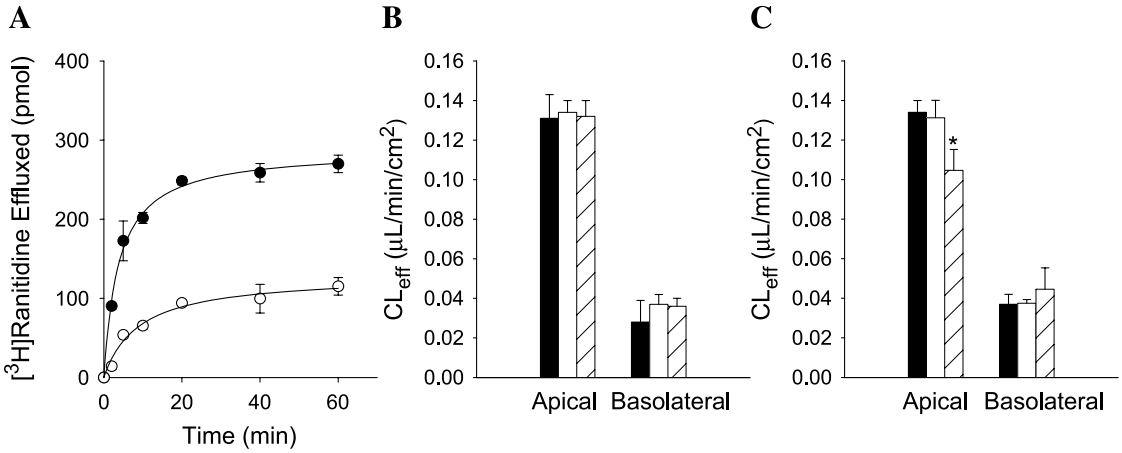


**Fig. 6.** Efflux of [$^3$H]ranitidine from preloaded Caco-2 cell monolayers. (A) Time course of [$^3$H]ranitidine efflux into AP (●) and BL compartments (○). [$^3$H]Ranitidine (0.1 mM; 0.75 μCi/mL) was loaded into Caco-2 cells from the AP side for 30 min. Cell monolayers were subsequently washed three times with 4°C transport buffer and the amount of [$^3$H]ranitidine effluxed into AP and BL compartments evaluated at 37°C as a function of time was quantified. (B) Effect of intracellular concentration on AP and BL efflux of [$^3$H]ranitidine from preloaded Caco-2 cell monolayers. [$^3$H]Ranitidine (0.75 μCi/mL) was loaded into Caco-2 cells at 1 μM (solid bars), 100 μM (open bars), and 1000 μM (hatched bars) for 30 min. Calculated initial intracellular concentrations (calculated from experimentally determined intracellular mass and previously reported cell volume) were 2.45 ± 0.23, 238.9 ± 47.0, and 1371 ± 108 μM, respectively. Cell monolayers were subsequently washed three times with 4°C transport buffer and the amount of [$^3$H]ranitidine effluxed into AP and BL compartments measured over 5 min at 37°C. $CL_{eff}$ was determined in the linear range of efflux (5 min) and calculated as described in "Data analysis." (C) *Trans* stimulation/inhibition of [$^3$H]ranitidine AP and BL efflux from preloaded Caco-2 cell monolayers. [$^3$H]Ranitidine (0.1 mM; 0.75 μCi/mL) was loaded into Caco-2 cells from the AP side for 30 min. Cell monolayers were subsequently washed three times with 4°C transport buffer and the amount of [$^3$H]ranitidine effluxed into AP and BL compartments in the absence (solid bars) or presence of 2 mM AP ranitidine (open bars) or 2 mM BL ranitidine (hatched bars) over 5 min at 37°C was quantified. $CL_{eff}$ was determined in the linear range of efflux (5 min) and calculated as described in "Data analysis." All experiments were conducted at an AP and BL pH of 7.2. Data represent mean ± SD; $n$=3. * $p$<0.05 compared to $CL_{eff}$ in the absence of extracellular ranitidine.

the ability of ranitidine to traverse the BL membrane after AP uptake, Caco-2 cells were preloaded from the AP side with [$^3$H]ranitidine (0.1 mM; 0.75 μCi/mL) for 30 min (sufficient to achieve steady-state intracellular levels; see Fig. 1B, inset). Preloaded cell monolayers were washed after loading and the efflux of preloaded [$^3$H]ranitidine was evaluated into both AP and BL chambers as a function of time. Efflux of [$^3$H]ranitidine from preloaded Caco-2 cells was relatively rapid into both AP and BL compartments (Fig. 6A). Total efflux to the AP chamber at 60 min was approximately 2.5-fold greater than that observed to the BL chamber indicative of apically directed secretion (e.g., P-gp) (Fig. 6A). Negligible [$^3$H]ranitidine remained inside the cells (~2.5% of initial) at the conclusion of the experiment suggesting that nearly complete efflux was achieved.

Two strategies were utilized to investigate the potential mechanism of BL efflux of ranitidine (i.e., carrier-mediated or diffusive). To determine if the BL efflux was saturable, the BL efflux rate was measured as a function of concentration by preloading Caco-2 cells with increasing concentrations of [$^3$H]ranitidine (1.0, 100, and 1000 μM) to achieve a wide range of intracellular concentrations. Because the loading process is inherently nonlinear (ranitidine AP uptake $K_m$, 0.45 mM; Fig. 1B), intracellular levels did not increase linearly with the applied loading concentration. The calculated initial intracellular concentrations were 2.45 ± 0.23, 238.9 ± 47.0, and 1371 ± 108 μM, respectively. As a result, the efflux rate was normalized to the loaded initial intracellular concentration and presented as an efflux clearance ($CL_{eff}$). No significant differences were observed in the $CL_{eff}$ to either the AP or BL compartment over the concentration range examined (Fig. 6B), providing no evidence for a saturable efflux mechanism. The $CL_{eff}$ was significantly greater in the AP direction (Fig. 6B) consistent with the time course study and the presence of an apically directed secretory process for ranitidine. *Trans* stimulation studies have been used previously to provide evidence for the existence of a carrier-mediated mechanism (28,29). In such studies, stimulation of efflux by a compound applied on the *trans* (i.e., opposite) side of the membrane that shares the carrier is observed in cases where efflux is carrier mediated. Application of unlabeled ranitidine (2 mM) in either the AP or BL buffer failed to significantly stimulate the BL efflux of preloaded [$^3$H]ranitidine as measured by the $CL_{eff}$ (Fig. 6C, basolateral). These results thus provide no clear evidence for a carrier-mediated efflux process for ranitidine on the BL membrane. Interestingly, application of unlabeled ranitidine (2 mM) in the BL compartment reduced the AP [$^3$H]ranitidine $CL_{eff}$ (Fig. 6C, apical). No effect, however, was observed on the AP [$^3$H]ranitidine $CL_{eff}$ in the presence of unlabeled ranitidine (2 mM) in the AP compartment. The reduced AP $CL_{eff}$ may result from competition for apically directed secretion (e.g., P-gp) when excess ranitidine concentrations are present on the BL side of the monolayers.

## DISCUSSION

Previous investigations into the mechanism of ranitidine absorption clearly demonstrated saturable and inhibitable absorptive transport for ranitidine across Caco-2 cell monolayers (5). To reconcile the saturable transport mechanism with the reported predominantly paracellular nature of ranitidine absorptive transport (3), a novel mechanism was proposed in which the saturable nature of ranitidine's absorptive transport could be mediated via interaction between the cationic moiety of ranitidine and anionic residues within the paracellular space (5). This saturable paracellular process also would be subject to inhibition by other cationic molecules (5). An alternative hypothesis, which is consistent with the observed transport and inhibition data, proposes that ranitidine is translocated in the absorptive direction via a saturable carrier-mediated uptake process across the AP membrane followed by subsequent BL efflux. Such a hypothesis implies the presence of a transcellular component to the overall ranitidine absorptive transport. The current study has explored this hypothesis and provided evidence for the existence of carrier-mediated AP uptake and P-gp-mediated efflux mechanisms in the absorptive transport of ranitidine in Caco-2 cells.

Absorptive transport of ranitidine in the current study was saturable as a function of concentration, consistent with previous results (Fig. 1A) (5). Examination of the AP uptake kinetics also indicated that a saturable process was involved in the uptake of ranitidine into the cells (Fig. 1B). Furthermore, ranitidine uptake was significantly reduced at 4°C and was linear with respect to concentration (Fig. 1B). These findings suggest that ranitidine is accumulated from the AP side of Caco-2 cells and that this uptake is mediated by saturable processes. Interestingly, the affinity (e.g., $K_m$) of ranitidine for the saturable transport and AP uptake processes were remarkably similar. The $K_m$ estimated for AP uptake of ranitidine was 0.45 ± 0.09 mM and in the same range as those estimated for overall ranitidine absorptive transport in both the current (0.27 ± 0.04 mM) and previous study (0.48 ± 0.11 mM) (5). The similar $K_m$ values may suggest that saturable uptake of ranitidine drives the overall saturable transport kinetics and that saturable uptake is the rate-limiting step in the absorptive transport of ranitidine.

Inhibition of both AP uptake and overall absorptive transport by various organic cations provides additional evidence for the involvement of specific carrier-mediated processes in the absorptive transport of ranitidine. The uptake process was significantly inhibited by a range of structurally distinct organic cations including small, permanently charged cations such as TEA and $MPP^+$, the lipophilic weak base quinidine, and the hydrophilic cation famotidine (Fig. 3A). These compounds also significantly reduced the overall transepithelial transport of ranitidine (Fig. 3B) suggesting that the inhibition of uptake results in the inhibition of overall transport across the cell monolayers. It does seem that the inhibition of ranitidine uptake and transport is influenced by specific structural features of the cationic compounds and does not result simply from the nonspecific presence of a positive charge. A wide range of potencies observed for inhibition of ranitidine uptake (Fig. 4) as well as the striking difference in inhibition observed by structurally similar TEA and TMA (Fig. 3) support this assertion. It is not clear from these experiments, however, if the reduction in ranitidine AP to BL transport can be solely explained by the reduced uptake or if some inhibition also occurs in the paracellular space. Fluorescent derivatives of

related $H_2$ receptor antagonist famotidine display saturable and inhibitable transport kinetics and a nearly exclusive paracellular distribution suggesting some inhibition also may occur within the paracellular space for this class of compounds (30).

The current study clearly implicates a role for a carrier-mediated transcellular transport in the absorptive transport of ranitidine in Caco-2 cells. Such a finding contradicts previous reports that concluded that ranitidine traversed Caco-2 cells via a predominantly paracellular mechanism (3,4,31). The evidence for a paracellular mechanism stems largely from the observed increased ranitidine flux under conditions that disrupt the tight junction (3,4). However, these studies do not provide information on the mechanism/route of ranitidine transport under "normal" conditions when the tight junction is sealed. Rather, they suggest only that the absorptive transport of ranitidine is sufficiently inefficient such that the tight junction provides a barrier to its overall transport. It is likely that absorptive transport of compounds such as ranitidine across Caco-2 cell monolayers occurs via parallel processes including both transcellular and paracellular transport. Kinetic modeling of ranitidine AP uptake and absorptive transport in Caco-2 cells under normal transport conditions supports this hypothesis of a contribution from both the paracellular and transcellular pathways [see accompanying paper (50)].

The results also demonstrate a role for P-gp in both the absorptive and secretory transport of ranitidine. P-gp was previously implicated in the transport of ranitidine in Caco-2 cells although the increase observed in absorptive transport of ranitidine in the presence of P-gp inhibitor verapamil (0.1 mM) was small and statistically insignificant (4). The authors suggested that this was due to the predominantly paracellular absorptive transport of ranitidine and limited ability of ranitidine to cross the AP membrane (31). Results in the present study clearly show that ranitidine is taken up across the AP membrane via a saturable mechanism (Fig. 1B) and that P-gp significantly attenuates ranitidine absorptive transport (Fig. 2). Inhibition with the potent P-gp inhibitor, GW918 (1 μM), results in both increased absorptive permeability as well as increased intracellular ranitidine concentrations (Fig. 2). Verapamil is neither a specific nor potent P-gp inhibitor, and the relatively high concentration required to inhibit P-gp (100 μM) may also nonspecifically inhibit the uptake of ranitidine in Caco-2 cells. Results with another nonselective P-gp inhibitor, quinidine, demonstrate a dramatically reduced uptake of ranitidine at relatively high quinidine concentrations (Fig. 3). We hypothesize that the observed effect (increased accumulation, decreased accumulation, or no net effect) of dual inhibition of uptake and efflux transporters will be dependent upon the relative affinity of the inhibitor for each transporter ($IC_{50}$ or $K_i$), the affinity of the substrate for the transporters ($K_m$), and the relevant extracellular and intracellular concentrations of both substrate and inhibitor. The P-gp inhibitor utilized in the present study, GW918, is very potent in its inhibition of P-gp and thus requires low concentrations (1 μM) that are less likely to affect the uptake process. Therefore, its effect on the P-gp-mediated efflux of ranitidine is not masked as may be the case for verapamil (Fig. 2B). Recently, GW918 also has been shown to inhibit the efflux transporter BCRP (32) and cimetidine has recently been identified as a BCRP substrate (33) suggesting that the GW918 effect on ranitidine transport may not be limited to P-gp. However, the extremely low level of BCRP expression in Caco-2 cells (34) suggests that the increased ranitidine absorptive transport in the presence of GW918 is virtually entirely due to inhibition of P-gp-mediated efflux.

Characterization of the ranitidine uptake mechanism at the AP membrane of Caco-2 cells revealed a marked sensitivity to inhibition by various organic cations (Figs. 3 and 4). Several recent studies have documented the expression of polyspecific transporters for organic cations in Caco-2 cells including those of the organic cation transporter (OCT1, OCT2, OCT3) and novel organic cation transporter (OCTN1 and OCTN2) families (27,35–37,51). Although the subcellular localization of all of these transporters in Caco-2 cells has not been elucidated, it seems that human organic cation transporter hOCTN2 is localized to the AP membrane (37). However, prototypical hOCTN2 substrate L-carnitine (1 mM) at a concentration much greater than its reported $K_m$ value [14 μM; (37)] displayed only ~10% inhibition of ranitidine uptake (Fig. 3) suggesting hOCTN2 may only play a minor role in ranitidine uptake. Similarly, the lack of inhibition by L-carnitine would presumably preclude a significant role for hOCTN1 in the uptake of ranitidine in Caco-2 cells. Significant inhibition of ranitidine uptake, however, was observed with the prototypical OCT substrates TEA and $MPP^+$ (Figs. 3 and 4). Despite a BL localization of such transporters in kidney and liver, recent investigations have demonstrated the lack of functional TEA-sensitive OCTs on the BL membrane of Caco-2 cells (16,35). Instead, functional evidence for OCT or OCT-like transporters on the AP membrane of Caco-2 cells has been suggested and the immunolocalization of hOCT1 to the AP membrane of Caco-2 cells has been demonstrated (27,51). Our studies with individually expressed hOCTs in *Xenopus* oocytes have demonstrated that ranitidine is a substrate for hOCT1 and has much weaker substrate activity toward hOCT2 (7). These findings potentially implicate OCTs such as hOCT1 or others with similar sensitivity to TEA/$MPP^+$ as candidates for mediating the uptake of ranitidine in Caco-2 cells. It is important to note that although mRNA expression of hOCT1 has been demonstrated in human small intestine (29), the hOCT1 intestinal protein expression level and subcellular localization remain unclear. Further studies are thus required to extend the observations of OCT-like transport activity in Caco-2 cells to the human intestine.

The total and saturable AP uptake of ranitidine was found to be strongly pH dependent (Fig. 5). A similar pH dependence (reduced uptake at acidic pH) also has been observed for cloned OCTs (rOCT1; rOCT2; hOCT3) transfected in MDCK and HeLa cells (38,39), and is thus consistent with a potential OCT-mediated absorption mechanism in Caco-2 cells. If the pH dependence observed in the *in vitro* Caco-2 system is operable in the human intestine, the physiological significance of such a mechanism in the intestine would presumably result in lower carrier-mediated absorption from proximal sites (i.e., duodenum) than from distal sites (i.e., ileum) where the intestinal pH is higher. Interestingly, the absorption of ranitidine from the terminal ileum of rat small intestine is quite extensive and higher than that observed from duodenum and midgut (40). Additionally,

deconvolution analysis of the absorption data for coadministered ranitidine and famotidine in humans demonstrated that a significant decrease in the oral absorption of famotidine occurred exclusively in the later stages of absorption when administered in the presence of ranitidine (41). These results suggest that modulation of intestinal transport by pH and/or differences in transporter expression levels may play an important role in the site-dependent absorption of ranitidine and similar hydrophilic cations. However, it will be of importance to clearly define the identification and expression of similar pH-dependent OCTs in the human intestine before such mechanistic observations can be extended to the human situation.

In addition to the observed pH dependence, AP uptake of ranitidine was independent of extracellular $Na^+$ concentration and dependent on the inside negative membrane potential (Table I). Such properties also are characteristic of the cloned OCTs (42) and support the role for an organic cation transport mechanism in the AP membrane of Caco-2 cells. The effect of membrane potential on saturable ranitidine uptake, however, was not as dramatic as that typically observed for permanently charged organic cation substrates such as TEA or $MPP^+$ in *Xenopus* oocytes injected with OCT cRNA (43). Several factors may contribute to this behavior including the impact of a permanent cationic charge on membrane potential, differences in depolarizing buffer conditions, and inherent differences between oocytes and cultured cells to regulate their own membrane potential. Relatively modest reductions in $MPP^+$ uptake (~30%) in Caco-2 cells under depolarizing conditions have been reported suggesting the effect of membrane depolarization in Caco-2 cells may not be as strong as that observed in other cells (27).

Carrier-mediated transport requires not only uptake across the AP membrane but also transport through the cytoplasm followed by subsequent efflux across the BL membrane. A previous study examining cimetidine absorption in rat small intestine concluded that the transcellular absorption of cimetidine was limited by an inability to exit across the BL membrane thus resulting in an exclusively paracellular route of absorption (44). In contrast, the present studies in Caco-2 cells clearly demonstrate the ability of ranitidine to exit across the BL membrane suggesting the transcellular route does play a role in overall ranitidine transport (Fig. 6A). Efflux of ranitidine across the AP membrane, however, was significantly greater and is consistent with a P-gp-mediated efflux process (Fig. 6A). This result may suggest that carrier-mediated mechanisms are directed only to the AP side or that the efficiency of carrier-mediated efflux transport is greater in the AP direction than in the BL direction. To address the mechanism of BL efflux, both saturation and *trans*-stimulation studies were utilized. No evidence was obtained for a carrier-mediated BL efflux mechanism under the experimental conditions employed in this study (Fig. 6B, C). This may reflect a low affinity of ranitidine for the BL efflux mechanism (below $K_m$) or the possibility that BL efflux is mediated via passive diffusion. Differences in membrane composition between AP and BL membranes have been noted in polarized cells and result in a more rigid and ordered AP membrane structure (45). It is possible that passive diffusion of ranitidine is greater across the BL membrane than the AP membrane as has been suggested for compounds in other studies (46,47). Interestingly, AP efflux of ranitidine also was not saturated over the concentration range examined (Fig. 6B) suggesting ranitidine is a relatively low affinity substrate of P-gp.

The studies presented here have elucidated a putative absorptive transport mechanism for the hydrophilic cationic drug, ranitidine, in the Caco-2 cell model of intestinal epithelium. Caco-2 cells have been well characterized with respect to transporters and enzymes. At confluence, these cells exhibit similar expression and localization for many transporters (e.g., PepT1, P-gp, etc.) as that found in the human intestine. However, differences in regard to expression of some transporters and tight junction permeability in the paracellular space between Caco-2 cells and human intestine have been documented (48,49). It should thus be clear that the studies reported here have defined the mechanism for absorptive transport of ranitidine in the Caco-2 cell model. Whereas these mechanistic studies do not necessarily prove a similar mechanism operable in human intestine, the results do provide a basis to design and conduct *in vivo* studies in humans to more clearly define the human intestinal absorption mechanism for ranitidine and similar hydrophilic organic cations.

In summary, the studies undertaken here provide strong evidence for the existence of a saturable AP uptake process for ranitidine in Caco-2 cells. The observed inhibition of uptake and overall absorptive transport of ranitidine by organic cations, increased absorptive transport of ranitidine after P-gp inhibition, and significant BL efflux collectively suggest a role for carrier-mediated transcellular transport in the absorptive transport of ranitidine. Ranitidine uptake across the AP membrane exhibits characteristics of OCT-mediated uptake. It is possible that one of the known OCT transporters or a novel transporter with OCT-like characteristics facilitates ranitidine AP uptake in Caco-2 cells. If these results can be extended to the *in vivo* situation, the identification of a carrier-mediated intestinal absorption mechanism for ranitidine and other $H_2$ receptor antagonists may explain their relatively good absorption in humans and has clear implications for potential drug–drug and drug–food interactions in the oral absorption of similar hydrophilic cations.

## ACKNOWLEDGMENTS

David L. Bourdet was supported by a Pharmaceutical Research and Manufacturers of America (PhRMA) Foundation predoctoral fellowship in Pharmaceutics. The Caco-2 cell line was kindly provided by Drs. Mary F. Paine and Paul Watkins of the University of North Carolina at Chapel Hill. GW918 and [$^3$H]ranitidine were kindly provided by GlaxoSmithKline.

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