The Contribution of Intestinal Secretion to the Dose-Dependent Absorption of Celiprolol

Shiu-Ming Kuo,[1,2] Brian R. Whitby,[3] Per Artursson,[4] and John A. Ziemniak[1–5]

Received January 12, 1993; accepted October 30, 1993

The contribution of the intestine to the nonlinear absorption of celiprolol in the rat was studied. After intravenous administration of ^{14}C-celiprolol to bile duct-cannulated rats, approximately 9% of the dose was found to be associated with intestinal tissue and its contents. Microhistoautoradiography of frozen intestinal sections showed a time-dependent secretion of celiprolol from the blood into the lumen of the rat intestine. Propranolol, a lipophilic β-blocker, was also found to be secreted into the intestine *in vivo* and transported in epithelial cells in both a temperature- and a pH-dependent manner, although to a lesser extent than celiprolol. Consistent with the observations in rats, transport of celiprolol from the basal-lateral to the apical side was found to dominate apical-to-basal transport using human Caco-2 cell monolayers. Additionally, using isolated rat small intestinal epithelial cells, celiprolol was found also to have a time- and temperature-dependent uptake, suggesting the involvement of a carrier-mediated system in its uptake. The uptake was inhibited by 2 m*M* celiprolol and propranolol and was also found to be pH dependent. Saturation of the carrier-mediated secretion of celiprolol in the intestine may result in enhanced absorption of celiprolol at high doses and account for its observed nonlinear absorption.

KEY WORDS: celiprolol; intestinal secretion; β-blockers; nonlinear absorption.

INTRODUCTION

Celiprolol (*N'*-[3-acetyl-4[3-[1,1-dimethylethyl)amino]-2-hydroxypropoxy]phenyl]-*N,N*-diethylurea) belongs to a new class of hydrophilic cardiac-selective β-blockers. In a series of rat studies, celiprolol was found to exhibit dose-dependent absorption, however, the decreased bioavailability noted at low oral doses cannot be readily explained by altered dissolution, first-pass metabolism, or changes in excretion. It was concluded from the experimental data that the intestine itself was the limiting organ responsible for the nonlinear increases in the bioavailability of celiprolol (1). This paper further investigates the cellular mechanisms responsible for the nonlinear absorption of celiprolol.

[1] Department of Drug Disposition, Rhône-Poulenc Rorer Central Research, Collegeville, Pennsylvania 19426.
[2] Present address: Nutrition Program, State University of New York, Buffalo, New York 14214.
[3] Hazleton, UK, North Yorkshire, England.
[4] Department of Pharmaceutics, Uppsala University, Uppsala, Sweden.
[5] To whom correspondence should be addressed at Rhône-Poulenc Rorer, 500 Arcola Road, NW13, Collegeville, Pennsylvania 19426–0107.

MATERIALS AND METHODS

Materials

DL-[^{14}C]Celiprolol (31.8 μCi/mg) and unlabeled DL-celiprolol were obtained from Rhône-Poulenc Rorer Pharmaceuticals Inc. Previous studies (1) suggest that the bioavailabilities of the individual enantiomers were similar, therefore all subsequent experiments utilized the racemic mixture due to its availability. The radiochemical and chemical purity of [^{14}C]celiprolol was 97 and 95%, respectively. The chemical purity of unlabeled celiprolol was greater than 98%. DL-[4-^{3}H]Propranolol hydrochloride in ethanol (89 mCi/mg, carrier free) was purchased from Amersham Corp. The chemical purity of [^{3}H]propranolol was greater than 99%. Unlabeled propranolol obtained from Sigma Chemical Co. was used to achieve the desired drug concentration and/or specific activity.

Male Sprague–Dawley rats weighing between 300 and 400 g were used for all *in situ* and *in vivo* studies. For the preparation of intestinal cells, rats with body weights in the range of 180 to 250 g were used to ensure consistent cell recovery. Prior to experimentation all rats were fasted overnight with free access to water. Studies requiring surgical modification were conducted using general anesthesia, and rats were euthanized at the termination of all experiments. The rats used for the collection of intestinal cells were sacrificed by pentobarbital overdose prior to intestine sampling.

Intestinal Absorption *in Situ*

A segment of rat small intestine (about 10 cm) was ligated at both ends with surgical silk. Approximately 1 mL of 20 mg/mL [^{14}C]celiprolol in physiological saline was administered into the lumen of the intestinal segment through a polyethylene cannula. The mesenteric vein draining the blood supply of this ligated segment was cannulated prior to the dosing with medical-grade Silastic tubing (0.02-in. I.D., 0.037-in. O.D.). Efferent blood from the cannula was continuously collected into heparinized tubes for approximately 1 hr in 600-μL fractions. Blood lost was replaced by a jugular infusion of a mixture of saline and heparinized blood freshly drawn from a donor rat. The ligated intestinal segment plus its contents were collected at the end of the experiment.

Intestinal Secretion After i.v. Dose

Bile duct-cannulated rats were used for this study. The entire intestine was ligated with surgical silk to form multiple (N = 12) 7-cm segments starting from the pylorus and continuing to the rectum. An intravenous dose (10 mg/kg) of either [^{14}C]celiprolol or [^{3}H]propranolol was administered through a precannulated jugular vein. Bile samples were collected continuously for the entire length of the experiment. Systemic blood samples, obtained by cardiac puncture, were taken prior to euthanasia. Urine collected in the bladder, as well as the intestine segments (including their contents and saline washes), was also collected at the completion of the experiment.

Total radioactivity in plasma, urine, bile, and intestinal contents was measured by liquid scintillation counting. In-

0724-8741/94/0500-0648$07.00/0

testinal tissues were solubilized with Soluene-350 (Packard Instrument) according to the manufacturer's instructions before being subjected to analysis. Selected efferent plasma samples from the *in situ* absorption studies were deproteinized by adding 2 vol of acetonitrile to 1 vol of sample. The resulting supernatant was then subjected to HPLC fractionation of [^{14}C]celiprolol and its ^{14}C-metabolites (1) using a Zorbax-CN column (15 cm × 4.6 mm) with a mobile phase of 10% methanol, 10% acetonitrile, and 10 m*M* dibutylamine phosphate at pH 5.6.

Microhistoautoradiography

Bile duct-cannulated rats were dosed intravenously with [^{14}C]celiprolol at 10 mg/kg (20 μCi per animal). At various time intervals after dosing, rats were sacrificed and a portion of duodenum, ileum, or colon was collected. The tissues were then thoroughly rinsed with physiologic saline. Approximately 10 mm of tissue, in duplicate, was placed in small molds containing Cryo-m-bed embedding compound (Bright Instrument Company Ltd., Huntingdon, Cambs, England). The embedded samples were kept at −70°C before transfer to a Bright's Rotary Rocker Microtome, which was kept at −16°C. Transverse sections (12 μm thick) were cut and loaded onto microscope slides which were precoated with Nuclear Emulsion (Type G5, Ilford Ltd., Mobberley, Cheshire, England). The slides were then exposed for various lengths of time before being photographically processed and stained with hematoxylin/eosin for visualization.

Preparation of Intestinal Cells and Characterization

The intestinal epithelial cells were prepared by a modification of the low-calcium method (2). All buffers used for the experiments contained, in addition to salts, 5 m*M* glucose, 2.5 m*M* glutamine, and antibiotics at 50 U of penicillin and 50 μg of streptomycin/mL.

A 30-cm segment of the small intestine was clamped at both ends by small hemostats and filled with pH 7.5 citrate buffer containing (m*M*) 75 NaCl, 4.7 KCl, 25 Na citrate, 1 KH_2PO_4, 25 $NaHCO_3$, and 0.5 dithiothreitol (DTT). The segment was then incubated in Leibovitz's L-15 medium for 20 min at 37°C. The contents of the segment were discarded and incubated with pH 7.5 EDTA buffer containing (m*M*) 110 NaCl, 4.7 KCl, 1 KH_2PO_4, 20 HEPES, 25 $NaHCO_3$, 1.2 $CaCl_2$, and 1.2 $MgSO_4$ for 30 min at 37°C. The resulting mixture was the pelleted down at 70*g* for 3 min. The resultant pellets were suspended in the working buffer in a plastic flask to a concentration of approximately 2 mg protein/mL. The viability of resuspended cells was determined by examining their ability to extrude trypan blue. On average, over 95% of freshly prepared cells extruded trypan blue. The preparations demonstrated maltase activity, a marker enzyme for the villus cells (3), between 1.14 to 2.40 U/min/mg protein.

Experiments to test the effect of pH gradient on drug uptake were carried out in buffers with 45 m*M* HEPES and no added $NaCHO_3$.

Uptake Studies of Isolated Intestinal Cells

The timed uptake of 50 μ*M* [^{14}C]celiprolol or [^{3}H]propranolol by freshly isolated intestinal cells was measured by a modification of the established rapid filtration method (4). In general, the working buffer containing the appropriate radioactive tracer was incubated in a polystyrene plastic tube in either a 37°C water bath or an ice bath (0°C). Cell suspensions were preincubated at the appropriate temperature and added to the reaction mixtures to initiate the reaction. To stop the reaction, ice-cold phosphate-buffered saline (154 m*M* NaCl, 3.4 m*M* KCl, 1.8 m*M* KH_2PO_4, 10 m*M* NaH_2PO_4 at pH 7.5) containing unlabeled propranolol at 0.5 mg/mL (or celiprolol at 2 mg/mL in the case of the inhibition experiment) was added to the tube. The inclusion of nonradiolabeled celiprolol in the stop solution was required to reduce nonspecific binding to the Millipore filter itself. The entire contents were then filtered through a 0.65-μm Millipore filter (DAWP), followed by three 3-mL washes of the same stop solution. The radioactivity associated with the filters was measured by liquid scintillation counting. Control experiments were conducted by eliminating the cells from the incubation mixture. The radioactivity associated with control experiments was subtracted from the total count to calculate activity per milligram of protein. The protein concentration of cell suspensions was measured by a modified Lowry method in the presence of sodium dodecyl sulfate (5).

Transepithelial Movement of ^{14}C-Celiprolol in Caco-2 Cells and Uptake Studies

The Caco-2 cell line was obtained from American Type Culture Collection, Rockville, MD. The cells were cultivated on polycarbonate filters (Costar Transwell cell culture inserts; mean pore diameter, 0.45 μm) as described previously (6,7).

All transport experiments were performed using 20 to 35-day-old monolayers in Hanks' balanced salt solution containing benzylpenicillin (100 U/mL), streptomycin (10 μg/mL), and 25 m*M* HEPES buffer. During the transport experiments, the monolayers were agitated on a microscope slide mixer. [^{14}C]Celiprolol was added to either the apical or the basolateral side of the monolayers, which were then incubated at 37°C and 95% relative humidity. Samples were withdrawn from the contralateral side of the monolayer at regular time intervals and subjected to liquid scintillation counting.

RESULTS

Limited Intestinal Absorption of Celiprolol *in Situ*

Isolated intestinal loop preparations were used to monitor celiprolol movement from the intestinal lumen to the mesenteric circulation. Although this procedure does not mimic the physiologic condition entirely, it does isolate the intestine from other organ systems while maintaining an intact blood circulation. The transcellular movement of celiprolol proceeded slowly, with the rate plateauing at approximately 30 min after dosing (Fig. 1). In a separate experiment, at an oral dose equivalent to 50 mg/kg, 75% of the administration radioactivity was recovered in the luminal washings and presumably represented unabsorbed drug. Another 23% of the radioactivity was found to be associated with the intestinal tissue itself and was not removed even after repeated luminal washings. Several mesenteric efferent plasma sam-

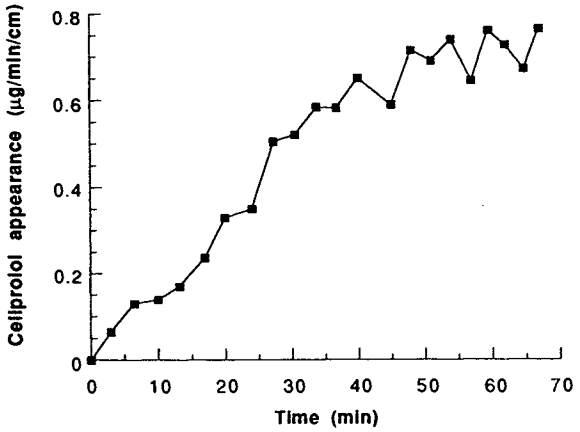

Fig. 1. The rate of [^{14}C]celiprolol appearance in the mesenteric efferent plasma after an intraluminal dose in the rat using an isolated intestinal loop.

ples obtained at different time points during the experiment were subjected to HPLC analysis of ^{14}C radioactivity. [^{14}C]Celiprolol was found to be the major (>95%) radioactive species present. These studies further illustrate the limited absorption of celiprolol and confirm that intestinal metabolism is not a major excretory pathway for celiprolol.

To investigate the existence of an intestine secretory process for celiprolol in the rat as a possible explanation of its low absorption, the intestine segment studies using bile duct-cannulated rats were conducted (Table I). At 4 hr after intravenous dosing, the radioactivity found in the systemic blood was less than 1 μg/mL, yet the total intestinal accumulation of radioactivity (luminal contents plus tissue) was equal to over 33 μg/g of tissue after celiprolol administration. This high tissue/blood ratio excludes the possibility of contamination due to the presence of blood and suggests an affinity of the rat intestine for celiprolol. Because the experiment utilized bile-duct cannulated animals as well as ligation of the intestine, the presence of radioactivity in the intestinal lumen after intravenous administration can be attributed only to some form of secretion of celiprolol across the intestinal tissue.

Propanolol was also tested to determine if the secretion of celiprolol was unique or was applicable to other β-blockers. Propranolol was selected since it represents a class of highly lipophilic β-blockers and its intestinal absorption is considered to be completely via diffusion (8). Although significantly less propranolol was associated with the intestine and its contents compared to celiprolol, the total radioactivity accounted for about 5.6 μg/g of tissue, while the plasma radioactivity of propranolol was 4 μg/mL.

Table I. Distribution of Radioactivity in Bile Duct-Cannulated Rats 4 hr After an i.v. Dose of [^{14}C]Celiprolol or [^{3}H]Propranolol

Dose	% of total dose[a]		
	Intestinal contents	Bile	Urine
Celiprolol (10 mg/kg)	8.8 ± 1.7	26 ± 6	25 ± 12
Propranolol (7.2 mg/kg)	1.2 ± 0.2	44 ± 20	15 ± 4

[a] Mean ± SD, with n = 4.

Multiple intestinal ligations were used in the previous study to determine whether any site-specific secretion of celiprolol (or propranol) was occurring. Celiprolol secretion was found to occur throughout the entire intestine from pylorus to rectum, with no evidence of site specificity. Greater than 30% of the radioactivity present in each segment (N = 12) was found to be associated with the intestinal tissue itself and not removed even after repeated luminal washings with saline. Only trace amounts of radioactivity (possibly due to blood contamination) were found in the stomach, suggesting that transport was not present in the upper gastrointestinal tract. Similar distribution patterns along the GI tract were also noted for propranolol, with no evidence seen of preferential secretion.

Based on the results of the intravenous intestinal secretion studies, microhistoautoradiography studies using frozen sections were also conducted in an attempt to locate the actual site of secretion within the intestinal mucosa and possibly determine the distribution of radioactivity at the cellular level. As shown in Fig. 2, sections from various sites in the intestine were collected at 20 min and 1, 2, and 4 hr after the i.v. dose. Although results from duodenum and ileum are reported here, similar results were observed in colonic sections collected from the same animals. At 20 min after an i.v. dose of 10 mg/kg [^{14}C]celiprolol, low levels of radioactivity began to appear in all intestinal tissues or tissue structures. At 1 and 2 hr postdose, radioactivity was associated mainly with the luminal mucus and the villus and goblet cells. At 4 hr postdose, radioactivity appeared predominantly in the intestinal lumen. These microhistoautoradiography studies revealed a time-dependent movement of radioactivity across the intestinal mucosa after intravenous dosing (mainly via villus and goblet cells) into the lumen. This is consistent with celiprolol undergoing some form of intestinal secretion; however, the exact intracellular localization of celiprolol could not be discerned from this study.

To understand the mechanisms that control intestinal transport, the time-dependent uptake of celiprolol in freshly prepared rat intestinal villi cells was also investigated. For the purpose of comparison, propranolol, a lipophilic β-blocker, was also studied. The results in Fig. 3 indicate that there is temperature-dependent uptake of celiprolol by the intestinal cells, which suggests the presence of nondiffusional transport. The 0°C uptake was much higher for propranolol, reflecting its higher lipophilicity, which possibly accounts for its high passive diffusion. Although the total uptake was higher in propranolol (500 pmol/mg protein), celiprolol exhibited a greater temperature-dependent uptake. Temperature-dependent uptake of celiprolol at 15 sec was reduced to 69 ± 11% (mean ± SD; n = 3) by the presence of 2 mM celiprolol in the incubation and to 66 ± 21% by the presence of 2 mM propranolol, suggesting that propranolol may also have an affinity for the same transport system.

Using intestinal membrane vesicles, uptake of other organic cations, N_v-methylnicotinamide and tetraethylammonium, was found to be pH dependent and to undergo proton–cation exchange in the kidney and liver (9–11). As shown in Fig. 4, the temperature-dependent uptake of celiprolol and

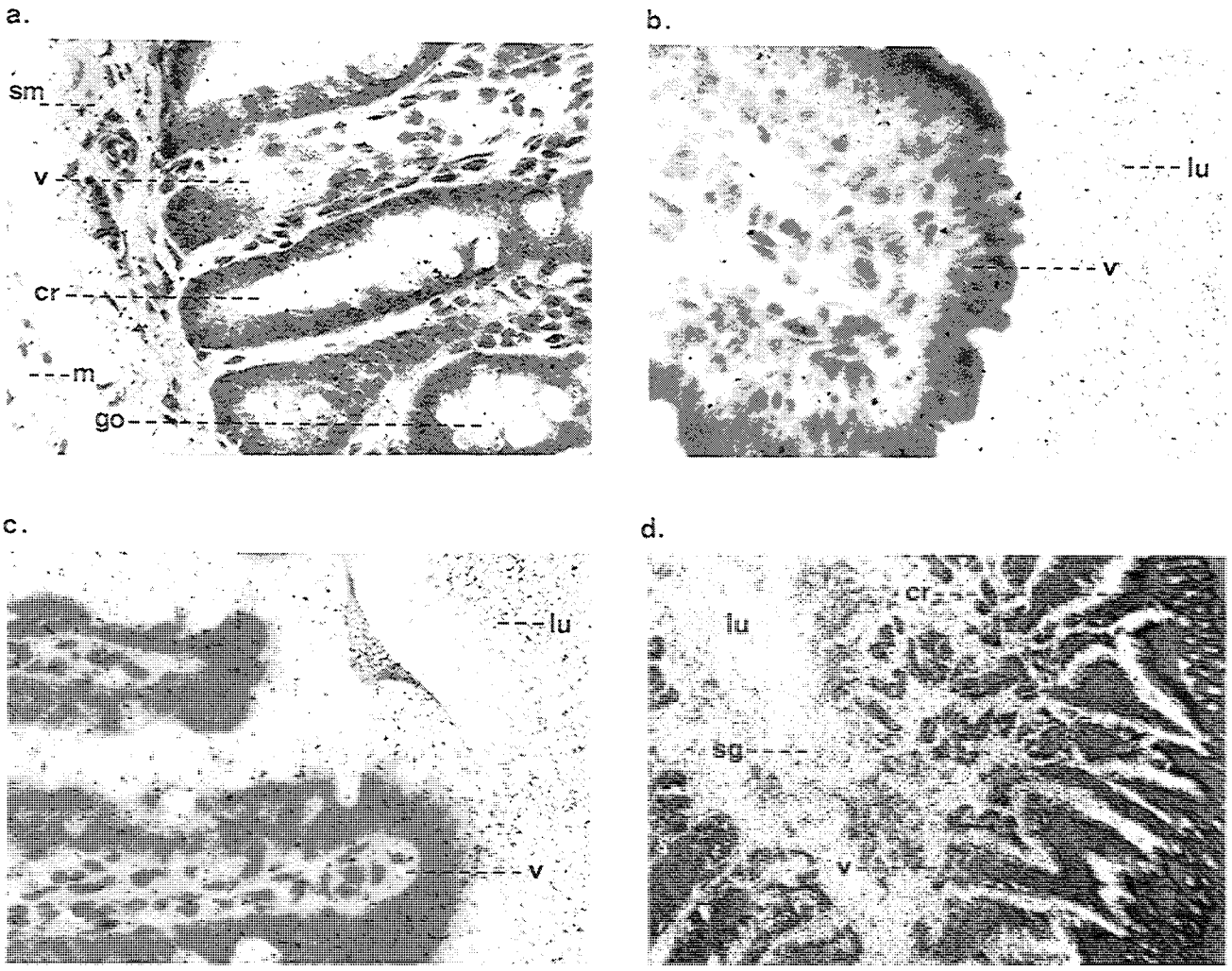

Fig. 2. Microhistoautoradiography of intestinal sections from the bile duct-cannulated rat (a) duodenum at 20 min, (b) duodenum at 1 hr, (c) duodenum at 2 hr, and (d) midileum at 4 hr after an i.v. dose of 10 mg/kg [^{14}C]celiprolol. cr, crypts; go, goblet cells; lu, lumen; mu, muscularis mucosa; sg, silver grains representing radioactivity; vi, villi. (a–c) ×400 and (d) ×63; reduced to 65% for reproduction.

propranolol was also pH dependent. Lowering the pH of the medium resulted in diminished uptake, while a higher pH increased the uptake of both agents. These observations are consistent with the possible presence of a β-blocker–proton exchanger or β-blocker–hydroxyl cotransport system. The effect of pH on propranolol uptake became insignificant at 5 min, probably because passive diffusion predominates at longer incubation times (Fig. 4).

Secretion of Celiprolol Across Human $CaCO_2$ Monolayers

Human Caco-2 cell monolayers were used to test whether the intestinal secretory system identified for celiprolol in rats may also be present in human tissue. Fluxes for celiprolol were found to occur in both directions: apical-to-basolateral as well as basolateral-to-apical sides (Fig. 5). Basolateral-to-apical transport was found to be approximately fivefold greater than the reverse flux at the 2-hr time point. This observation further confirms the ability of celiprolol to undergo intestinal secretion but also showed that the mechanism may be present in the human intestine as well.

DISCUSSION

The various *in vivo* studies conducted confirm that intestinal secretion is an important excretory pathway for celiprolol in rats while also suggesting that some carrier-mediated absorption may also be occurring. The overall net secretion of celiprolol in the intestinal mucosa could serve to limit its absorption and may account for its low oral bioavailability, especially at low doses. Saturation of this secretory pathway at higher doses may account for the nonlinear absorption of celiprolol.

From the microhistoautoradiography and cellular studies, it appears that celiprolol undergoes intestinal secretion via a carrier-mediated process. The autoradiographs confirm the net movement of celiprolol across the intestinal mucosa after intravenous administration. Based on the autoradiographs and the large amount of radioactivity associated with the lumen, drug accumulation in the lumen apparently occurred against a concentration gradient. This suggests the presence of carrier-mediated transport system in the apical membrane, although the presence of transport systems on

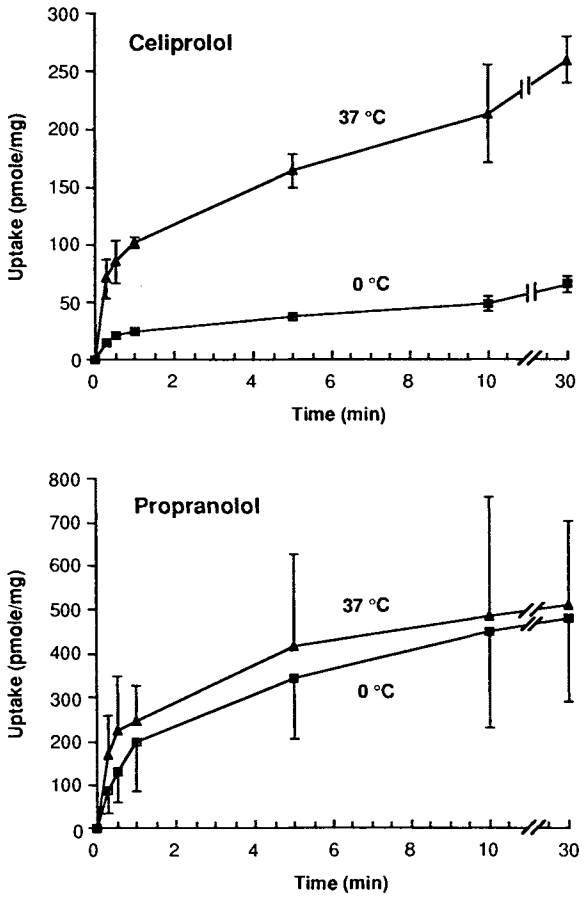

Fig. 3. The time- and temperature-dependent uptake of celiprolol and propranolol by freshly isolated rat small intestinal cells at pH 7.5/7.5 (cell/medium). Each point represents the mean ± SD of experiments on three intestinal preparations conducted in triplicate. Uptake were normalized for protein concentration.

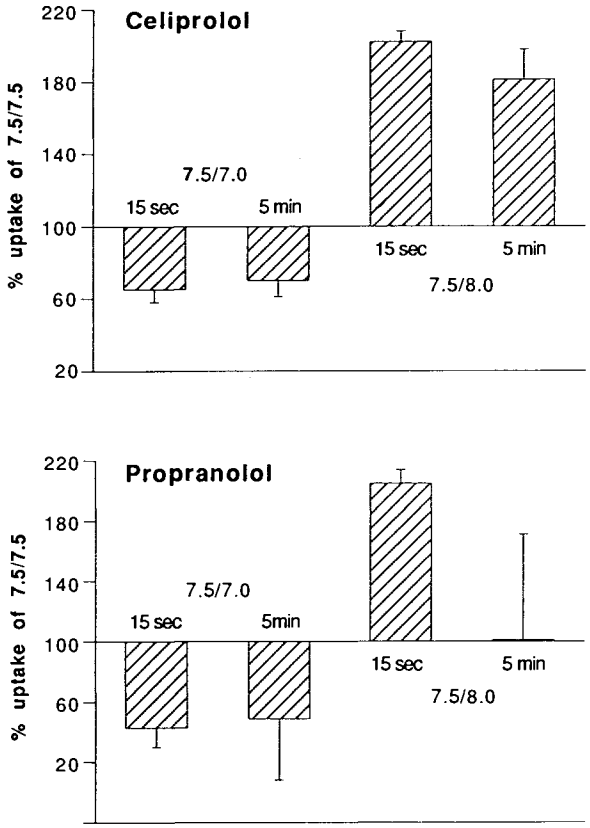

Fig. 4. The pH dependence of temperature uptake of celiprolol and propranolol by freshly isolated rat small intestinal cells. Each point represents the mean ± SD of experiments on three intestinal preparations conducted in triplicate. Temperature-dependent celiprolol uptake (defined as the uptake at 37°C minus the uptake at 0°C) at pH 7.5/7.5 (cell/medium) was 52 ± 6 and 142 ± 19 pmol/mg protein for 15 sec and 5 min, respectively. Temperature-dependent uptake of propranolol at pH 7.5/7.5 was 95 ± 20 and 82 ± 61 pmol/mg protein for 15 sec and 5 min, respectively.

both apical and basolateral membranes cannot be definitively ruled out.

Our results indicate that the secretory system involved may have an affinity for other β-blockers. Although propranolol also possesses a cationic charged group (pK_a, 9.5), its high lipophilicity (octanol/water partition coefficient = 39, compared to 0.2 for celiprolol) indicates that the hydrophobic portion of propranolol effectively neutralizes the charge. Our studies found that despite propranolol's ability to cross biological membranes by passive diffusion, there was still some detectable net intestinal secretion after intravenous dosing. Intestinal secretion of other β-blockers (acebutolol and pafenolol octanol/water, partition coefficients = 6.5 and 0.3, respectively) has also been reported (12,13). Combined with the results from this study, it seems likely that a transport system capable of secreting various β-blockers in the intestine likely exists.

The presence of a carrier-mediated secretory system for celiprolol in the intestine can be the basis for the reduction in bioavailability of celiprolol at low doses (14). The intestinal secretion of quaternary ammonium compounds has been studied in guinea pigs, and the role of saturable secretion on their nonlinear absorption outlined (15). A similar mechanism may also be present for celiprolol in the rat. At low doses, secretion of celiprolol into the intestinal tract serves to limit the absorption of celiprolol. As the secretion system begins to saturate overall absorption increases, resulting in nonlinear increases in bioavailability. Once secretion is totally saturated, passive diffusion dominates and a new equilibrium is reached with linear absorption now evident. This explanation is consistent with the rat bioavailability data for celiprolol previously reported (1).

Our studies utilizing epithelial intestinal cells suggest that celiprolol may also undergo some bidirectional transport. The cellular uptake of celiprolol using isolated intestinal cells was found to be both temperature and pH dependent, lending support for the presence of carrier-mediated transporter. The pH-dependent uptake of celiprolol cannot be explained simply by alterations in the ionization state of the drug as a function of pH. The 0°C uptake, which includes passive diffusion, did not change as the medium pH changed from 7.0 to 8.0. These results are consistent with the pK_a of celiprolol being 9.5. The strongest evidence acquired to date for carrier-mediated absorption of celiprolol is the ability of both celiprolol and propranolol to inhibit the cellular uptake of [^{14}C]celiprolol. The exact localization of the transport sys-

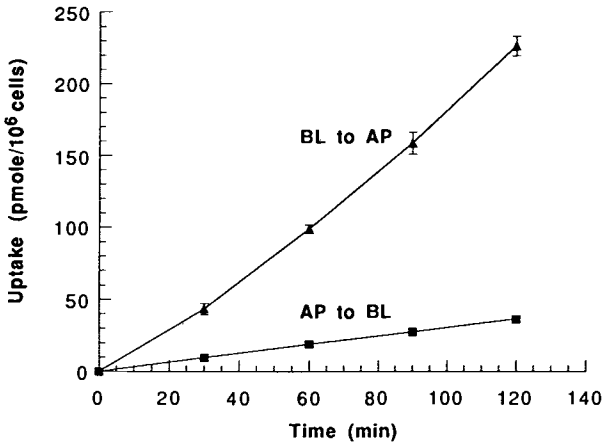

Fig. 5. Transmembrane movement of [^{14}C]celiprolol (10 μM) across Caco-2 cell monolayers. Each point represents the mean ± SD; n = 4. AP, apical; BL, basolateral.

tem in the epithelial cell membrane is presently unknown. It is also unknown whether one system or multiple systems are involved in the intestinal absorption/secretion of celiprolol.

Human studies have demonstrated increased absorption of celiprolol at elevated doses similar to that seen in rats (14). The use of the Caco-2 cell further confirms that celiprolol undergoes intestinal secretion and suggests that the same or a similar transport system identified in rats may also be present in humans. Further studies are required to confirm this hypothesis, but based on the results in Fig. 5, the Caco-2 cell line appears to be appropriate for further study of the intestinal transport of β-blockers.

The physiological role of this secretory pathway for celiprolol (and possibly other β-blockers) is presently unknown. Recently, it was proposed that the multidrug resistance gene product in the intestine acts as a defense system to prevent undesirable chemicals from entering the systemic circulation (16). This secretory system for β-blockers could be part of a natural defense system in the intestine designed to limit absorption.

ACKNOWLEDGMENTS

The authors would like to thank Dr. Roy Kickelbein, Yale University, for sharing information on intestinal cell preparation before publication and Bonnie Blume for help in manuscript preparation.

REFERENCES

1. S.-M. Kuo and J. Ziemniak. The physiological disposition of individual isomers of celiprolol—a new β-blocker. In *Proceedings for the Third International SCBA Symposium and Workshop,* 1990, p. 323 (abstr.)
2. U. Sundaram, R. G. Knickelbein, and J. W. Dobbins. PH regulation in ileum: Na^+-H^+ and Cl^--HCO_3_ exchange in isolated crypt and villus cells. *Am. J. Physiol.* **260**:G440–G449 (1991).
3. M. M. Weiser. Intestinal epithelial cell surface membrane glycoprotein synthesis. *J. Biol. Chem.* 248:2536–2541 (1973).
4. S. M. Grassl and P. S. Aronson. Na^+/HCO^{3-} co-transport in basolateral membrane vesicles isolated from rabbit renal cortex. *J. Biol. Chem.* **261**:8778–8783 (1986).
5. G. L. Peterson. Determination of total protein. *Methods Enzymol.* 91:95–119 (1983).
6. P. Artursson. Epithelial transport of drugs in cell culture. I. A model for studying the passive diffusion of drugs over intestinal absorption (Caco-2) cells. *J. Pharm. Sci.* 79:476–482 (1990).
7. P. Artursson and J. Karlsson. Correlation between oral drug absorption in humans and apparent drug permeability coefficients in human intestinal epithelial (Caco-2) cells. *Biochem. Biophys. Res. Comm.* 175:880–885 (1991).
8. D. C. Taylor, R. Pownall, and W. Burke. The absorption of β-adrenoceptor antagonists in rat in-situ small intestine; The effect of lipophilicity. *J. Pharm. Pharmacol.* 37:280–283 (1985).
9. M. Takano, K. Inui, T. Okano, H. Saito, and R. Hori. Carrier-mediated transport systems of tetraethylammonium in rat renal brush-border and basolateral membrane vesicles. *Biochim. Biophys. Acta* 773:113–124 (1984).
10. C. Rafizadeh, F. Roch-ramel, and C. Schali. Tetraethylammonium transport in renal brush border membrane vesicles of the rabbit. *J. Pharmacol. Exp. Ther.* **240**:308–313 (1987).
11. R. H. Moseley, J. Morrissette, and T. R. Johnson. Transport of N1-methylnicotinamide by organic cation-proton exchange in rat liver membrane vesicles. *Am. J. Physiol.* **259**:G973–G982 (1990).
12. C. F. George and B. S. Gruchy. Elimination of drugs by active intestinal transport. *J. Pharm. Pharmacol.* 31:643–648 (1979).
13. H. Lennernas and C. G. Regardh. Dose-dependent intestinal absorption and significant intestinal excretion (exsorption) of the beta-blocker pafenolol in the rat. *Pharm. Res.* **10**:727–731 (1993).
14. F. S. Caruso, H. D. Doshan, P. H. Hernandez, R. Costello, W. Applin, and E. S. Neiss. Celiprolol: Pharmacokinetics and duration of pharmacodynamic activity. *Br. J. Clin. Pract.* 39(Suppl. 40):12–16 (1985).
15. K. Turnheim and F. Lauterbach. Interaction between intestinal absorption and secretion of monoquarternary ammonium compounds in guinea pigs—a concept for the absorption kinetics of organic cations. *J. Pharmacol. Exp. Ther.* **212**:418–424 (1980).
16. S. Hsing, Z. Gatmaitan, and I. M. Arias. The function of Gp170, the multidrug-resistance gene product, in the brush border of rat intestinal mucosa. *Gastroenterology* **102**:879–885 (1992).