Mucosal Uptake of Gabapentin (Neurontin) vs. Pregabalin in the Small Intestine

Nusara Piyapolrungroj,[1] Cheng Li,[2] Howard Bockbrader,[3] Guangyu Liu,[4] and David Fleisher[4,5]

Received April 20, 2001; accepted May 1, 2001

Purpose. To compare the mucosal membrane transport of gabapentin and pregabalin in animal small intestine.

Methods. Uptake of the two drugs by brush-border membrane vesicles (BBMV) from rat and rabbit small intestine was studied as a function of temperature, uptake-medium sodium content, and intestinal region. Amino acid inhibition studies were conducted with pregabalin.

Results. Gabapentin uptake by rat and rabbit jejunal BBMV was sodium independent, whereas pregabalin uptake was sodium dependent. Uptake of both drugs in rabbit small intestinal vesicles was greater at 25°C than at 4°C in the absence of sodium and an additional increase in uptake was observed for pregabalin at 25°C in the presence of sodium. Pregabalin uptake in rabbit duodenal, jejunal, and ileal BBMV was equivalent, whereas gabapentin uptake was greater in duodenal and ileal BBMV, compared with jejunal BBMV. Although inhibition is weak, a decrease in BBMV uptake of pregabalin is observed with coincubation of high concentrations of both neutral and basic amino acids.

Conclusions. Amino acid carriers mediate the apical uptake of both drugs in the small intestine. Although gabapentin and pregabalin are structurally similar, their small intestinal mucosal uptake differs in sodium dependence and region dependence. Gabapentin uptake is likely mediated by system $b^{0,+}$, whereas pregabalin uptake is also mediated by B^0 and/or $B^{0,+}$.

KEY WORDS: gabapentin; pregabalin; intestinal absorption; membrane vesicles.

INTRODUCTION

The gabapentenoids (three substituted GABA derivatives) are under study for their clinical potential to treat a number of diseases of the central nervous system as a function of their anticonvulsant, antinociceptive, anxiolytic, and neuroprotective activity (1). Of this drug class, Neurontin (gabapentin [GP]) is on the prescription market in tablet, capsule, and liquid dosage forms, and Pregabalin (isobutyl GABA [IBG]) is currently under FDA review as a capsule dosage form (Fig. 1). Although these derivatives were initially synthesized to increase γ-amino butyric acid (GABA) lipophilicity to passively permeate the blood-brain barrier, it has been reported that their transport across this barrier is mediated by the large neutral amino acid carrier, system L (2). Consistent with saturation of carrier-mediated transport in other organ systems, clinical studies with GP have shown dose-dependent absorption, and intestinal uptake of GP was inhibited by several large neutral amino acids (3). In a study comparing the intestinal absorption of GP and IBG, cross-inhibition profiles of these drugs were not consistent with transport by a single carrier (4). The research reported in this follow-up communication was motivated by a recent study in canine small intestinal brush-border membrane vesicles (BBMV) showing that uptake of the nitric oxide synthase inhibitor, NG-nitro-L-arginine, is mediated by both sodium-dependent system $B^{0,+}$ and sodium-independent system $b^{0,+}$ amino acid carrier systems (5).

MATERIALS AND METHODS

Chemicals

Unlabeled and C^{14}-labeled GP (specific activity 4.3 mCi/mmol) and IBG (specific activity 3.6 mCi/mmol) were supplied by Pfizer, formerly Parke Davis/Warner Lambert. Analysis of vesicle uptake of these drugs by measurement of traced radiolabel is legitimized by the fact that these drugs are not metabolized by rat, rabbit, or human (6). Candidate transport inhibitors including L-phenylalanine, L-leucine, L-methionine, L-lysine, L-arginine, L-proline, L-glycine, β-alanine, γ-amino butyric acid (GABA), glycyl L-proline, and cephalexin were obtained from Sigma (St. Louis, MO). Mannitol, D-glucose, salts, and buffers as well as the alkaline phosphatase (ALP) and sodium-potassium ATPase assay kits were also obtained from Sigma. The protein assay kit was obtained from Bio-Rad (Richmond, CA). D-1-^{3}H glucose (specific activity 15.5 Ci/mmol), used to verify carrier-mediated vesicle uptake, was obtained from New England Nuclear (Boston, MA).

Intestinal Tissue

Rat jejunum was isolated as the first 40 cm of small intestine past the ligament of Trietz from anesthetized Sprague-Dawley (Charles River Breeding Labs, Pearl River, NY) rats. Rabbit intestine was obtained fresh from other laboratories immediately after animal anesthesia and removal of other organ systems. Most experiments were performed on jejunum removed up to 75 cm from the ligament of Trietz. In the regional studies, duodenum was obtained from the stomach to the ligament of Trietz and ileum up to 50 cm from the ileocecal junction. Typically, pooled jejunal samples from several rats were required for uptake experiments comparing treatments, whereas intestinal segments from one rabbit provided sufficient tissue for an equivalent number of treatment variables.

BBMV Preparation and Characterization

Vesicle preparation procedures with intestinal tissue were performed on ice or under refrigeration. Intestinal segments were washed with ice-cold saline, the mucosa was removed by scraping with glass cover slides, and the tissue was homogenized. Intestinal BBMV were prepared by the cal-

[1] Faculty of Pharmacy, Silpakorn University, Nakorn-pathom 73000, Thailand.
[2] Current address: Schering Plough Research, Kenilworth, New Jersey 07033-1300.
[3] Pfizer Global Research & Development, 2800 Plymouth Rd. Ann Arbor, Michigan 48106.
[4] College of Pharmacy, The University of Michigan, Ann Arbor, Michigan 48109-1065.
[5] To whom correspondence should be addressed. (e-mail: fleisher@umich.edu)

0724-8741/01/0800-1126$19.50/0

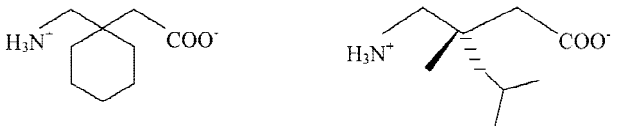

Fig. 1. Structures of Neurontin (Gabapentin [GP]) and Pregabalin (Isobutyl GABA [IBG]).

cium precipitation method as described by Yuasa *et al.* (7). Preparation protein determination for uptake normalization was determined by using the Bio-Rad assay kit. Brush-border membrane enrichment over intestinal mucosal homogenate was determined by measuring alkaline phosphatase activity.

Uptake Experiments

BBMV overshoot uptake of ^{3}H-D-glucose from 100 μM D-glucose traced with 2 μCi/mL of radiolabel was used to verify carrier-mediated vesicle uptake from incubation buffer composed of 80 mM Hepes, 45 mM Tris, and 100 mM NaCl adjusted to pH 7.5 and adjusted to isotonicity with mannitol. Vesicle loading buffers and sodium-free incubation buffers replaced NaCl with 100 mM of KCl and in some cases with 100 mM of choline chloride. Mannitol adjustment of isotonicity was altered in studies using high concentrations of drug uptake inhibitor candidates. Drug incubation concentrations were 1 mM in rat BBMV and 200 μM in rabbit BBMV. Uptake experiments were conducted by using a Millipore filtration apparatus (Millipore Corp., Bedford, MA). Typically, 40 mL of uptake solution was rapidly mixed with 10 mL of membrane vesicles (0.07–0.1 mg of protein). After incubation for a selected time interval, uptake was stopped with an ice-cold stop solution containing 100 mM mannitol, 100 mM KCl, and 10 mM HEPES at pH 7.5. Stopped uptake mixtures were filtered and washed with ice-cold stop buffer. Radioactivity remaining on the filter was determined by using a Beckman LS 6000 SC scintillation counter (Beckman Instruments, San Jose, CA). All uptake measurements were corrected for nonspecific filter binding.

Data Analysis

Uptake values in the rat BBMV and rabbit BBMV inhibition studies are mean values of duplicate uptake studies on the same preparation. Uptake values in all other rabbit BBMV studies are represented as the means ± SEM of three rabbit preparations using the average of duplicate uptakes from the same animal preparation. Significant differences between uptake values as a function of treatment variables are determined by either Student's *t* test or analysis of variance (ANOVA) where appropriate. Statistically significant differences from controls were assessed at a 95% level by one-factor ANOVA.

RESULTS

The specific activity of the brush-border marker enzyme, alkaline phosphatase, was increased in the vesicle preparations over that of crude intestinal homogenate corresponding to an enrichment factor of 13.7-fold in rat and 16.5-fold in rabbit. Sodium-potassium ATPase was not enhanced in the vesicle preparation (enrichment factor < 1 in both species), indicating minimal contamination with basolateral membranes. 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 membrane vesicles in preparations from both species.

Previous studies showing carrier-mediated intestinal transport of GP and IBG had been conducted in rat *in situ* intestinal perfusions and *in vitro* intestinal tissue (3,4). In a preliminary study to follow up this previous work in rats, average uptake from a single pooled animal preparation was determined as a function of incubation time using BBMV made from rat jejunum. This study showed that 1 mM GP uptake was sodium independent (Fig. 2a), whereas 1 mM IBG uptake included a sodium-dependent component (Fig. 2b). This study was repeated in rabbit jejunum using 200 μM drug concentrations. Tissue from one rabbit is sufficient to conduct region-dependent experiments that would require tissue from 6 to 10 rats. Similar to rats, jejunal uptake of GP was sodium independent (Fig. 3a), whereas uptake of IBG included a sodium-dependent component (Fig. 3b).

Comparing 30-s uptake at 4°C vs. 25°C in rabbit jejunal BBMV, uptake of IBG (Fig. 4a) showed a greater increase with temperature than did GP, and IBG uptake was significantly lower than GP uptake at 4°C (Fig. 4b). As a function of rabbit small intestinal region, IBG uptake at 30 s by BBMV was region independent (Fig. 4a), whereas GP uptake was lower in the jejunum than in the duodenum and ileum (Fig. 4b). In a single jejunal BBMV pregabalin uptake study, self-inhibition of 200 μM ^{14}C-labeled IBG by cold drug at 50 mM reduced labeled drug uptake by only 50% in BBMV from vesicle incubation solutions containing 100 mM of sodium chloride at pH 7.5. For dipolar (neutral) amino acid inhibitors, both 50 mM leucine and 50 mM phenylalanine as well as 50 mM GP reduced drug uptake by 35%, whereas 50 mM methionine reduced drug uptake by 25%. For basic amino acids, 50 mM arginine and 50 mM lysine reduced drug uptake by 25% and 20%, respectively. IBG uptake was not reduced by proline, glycine, GABA, glycyl L-proline, or cephalexin (Table I). Inhibition data were obtained at 30 s of vesicle incubation time.

DISCUSSION

Carrier-mediated transport was previously shown for both GP (3,4) and IBG (4) in rat small intestine. Cross-inhibition studies suggested that multiple amino acid transport systems might be involved in the small intestinal absorption of these gabapentenoids (4). A recent study (5), showing that both sodium-dependent and sodium-independent transporters mediated small intestinal brush-border membrane uptake of the nitric oxide synthase inhibitor, N^G-nitro-L-arginine (L-NNA), led to the research outlined in this short communication. Despite their structural similarity, the results presented here provide a clear indication that IBG uptake is sodium dependent, whereas GP uptake does not depend on medium sodium content in both rat and rabbit small intestinal BBMV. The higher uptake values obtained for both drugs in rat BBMV is partly due to the fivefold higher concentrations used compared with rabbit.

Based on the fact that GP and IBG are zwitterionic at intestinal pH, neutral amino acid carriers should mediate their intestinal absorption. This projection is consistent with previous inhibition studies (3,4). Although GP and IBG are

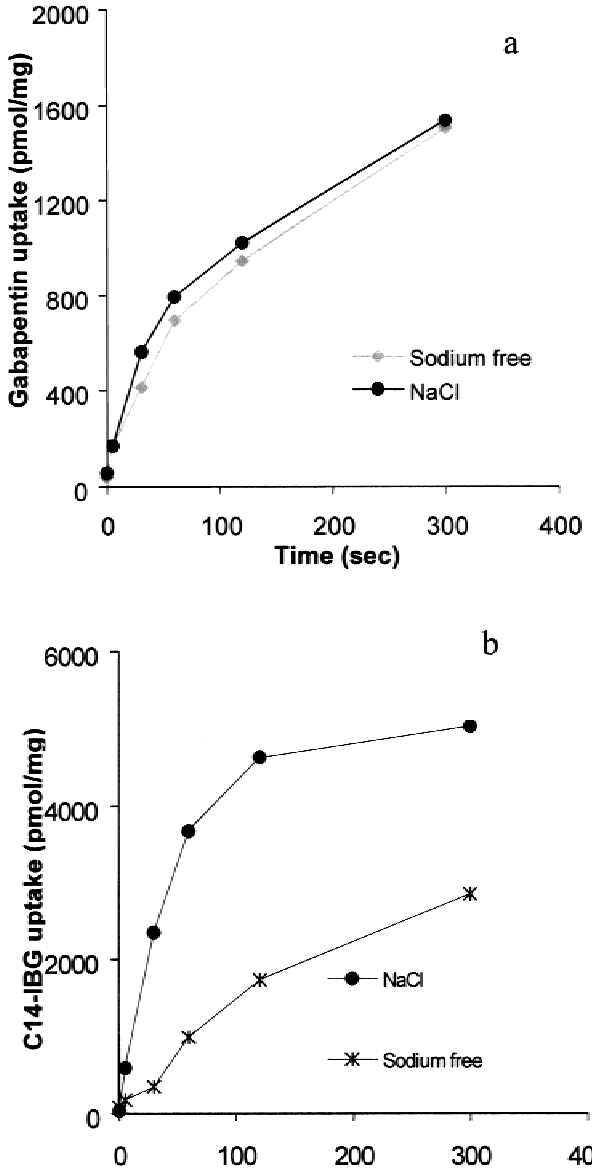

Fig. 2. (a) Gabapentin (GP) uptake as a function of time in rat jejunal BBMV in the presence and absence of 100 mM of sodium chloride in isotonic incubation media. Incubation drug concentrations are 1 mM traced with ^{14}C-GP. Uptake values are the average of duplicate measurements in the same rat BBMV preparation. (b) Pregabalin (IBG) uptake as a function of time in rat jejunal BBMV in the presence and absence of 100 mM of sodium chloride in isotonic incubation media. Incubation drug concentrations are 1 mM traced with ^{14}C-IBG. Uptake values are the average of duplicate measurements in the same rat BBMV preparation.

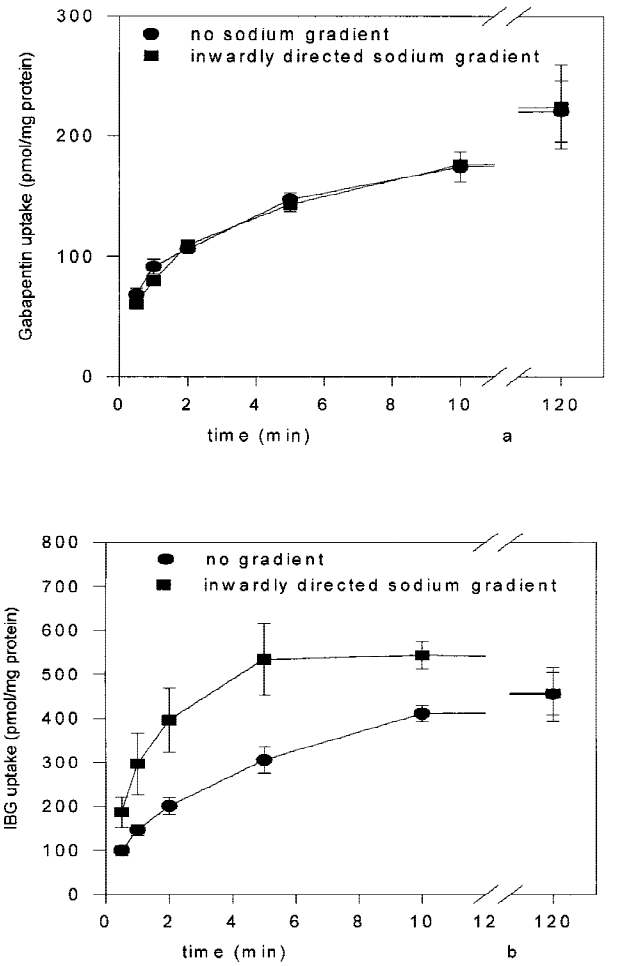

Fig. 3. (a) Neurontin (GP) uptake as a function of time in rabbit jejunal BBMV in the presence and absence of 100 mM of sodium chloride in isotonic incubation media. Incubation drug concentrations are 200 μM traced with ^{14}C-GP. Uptake values are shown as the mean value of three rabbit BBMV preparations ± SEM using the average of duplicate measurements in the each rabbit BBMV preparation. (b) Pregabalin (IBG) uptake as a function of time in rabbit jejunal BBMV in the presence and absence of 100 mM of sodium chloride in isotonic incubation media. Incubation drug concentrations are 200 μM traced with ^{14}C-IBG. Uptake values are shown as the mean value of three rabbit BBMV preparations ± SEM using the average of duplicate measurements in each rabbit BBMV preparation.

neutral amino acid-like drugs, another report has shown that GP inhibited arginine transport in Caco-2 cell monolayers (8). Intestinal carriers mediating the transport of neutral amino acids include sodium-dependent B^0 (ATB^0) for neutral amino acids, sodium-dependent $B^{0,+}$ ($ATB^{0,+}$) for neutral and basic amino acids, sodium-independent $b^{0,+}$ for neutral and basic amino acids, sodium-independent L (LAT2) for neutral amino acids, and y^+L which mediates sodium-dependent transport of neutral amino acids and sodium-independent transport of basic amino acids (9). However, glycoprotein dependence of $b^{0,+}$, y^+L, and LAT2, which are amino acid exchangers, indicate that y^+L and LAT2 are associated with the basolateral rather than brush-border membrane (10). This localization is somewhat surprising for intestinal system L because amino acid transport by LAT2 is enhanced by a proton gradient, a dependence that is typically observed for brush-border transporters in line with low mucosal microclimate pH (11).

In addition to determining uptake sodium dependence as a function of small intestinal region, carrier-mediated uptake was evaluated by studies conducted at 4°C and 25°C. Statistically higher uptake was observed for both drugs at the higher temperature; however, this was more dramatic for IBG, which showed much lower uptake at 4°C than GP. Although the differences at 4°C are consistent with a lower IBG partition coefficient for passive membrane permeation (log

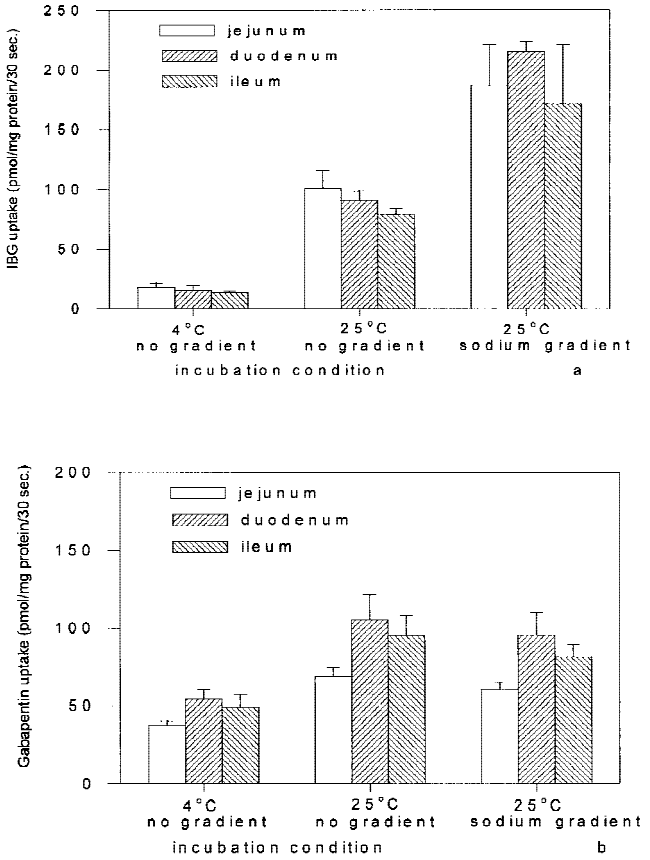

Fig. 4. (a) Means ± SEM pregabalin (IBG) uptake as a function of temperature, sodium-gradient, and intestinal region in rabbit small intestinal BBMV. Incubation drug concentrations are 200 μM traced with ^{14}C-IBG. Uptake at 25°C is statistically greater than at 4°C, and uptake in the presence of a sodium gradient is statistically greater than in the absence of a sodium gradient in each intestinal region. (b) Means ° SEM gabapentin (GP) uptake as a function of temperature, sodium-gradient, and intestinal region in rabbit small intestinal BBMV. Incubation drug concentrations are 200 μM traced with ^{14}C-GP. Uptake at 25°C is statistically greater than at 4°C in each intestinal region. Uptake in the jejunal BBMV is statistically lower than in duodenal and ileal BBMV at both temperatures and in the presence or absence of sodium.

K_p –1.10 for GP and –1.35 for IBG), the 4°C differences are larger than might be expected. The possibility that sodium-independent amino acid exchangers might be less sensitive to temperature than sodium-dependent amino acid cotransporters could explain the 4°C data, but there is no literature evidence for this.

Previous *in vitro* and *in situ* studies in rat intestine indicated that cross-inhibition between neutral amino acids, leucine, and phenylalanine, and these two gabapentenoids was weak. Weak inhibition of ^{14}C-IBG at 50 mM inhibitor concentration is also observed in these BBMV studies. Self-inhibition of labeled IBG is only 50%; inhibition by neutral amino acids and GP is only 25–35% and by basic amino acids only 20–25%. The possibility that system B^0 is partially involved in IBG transport might explain the slightly higher inhibition by neutral compared to basic amino acids.

Previous studies on rabbit small intestinal transport of neutral and basic amino acids indicated that transport increased from proximal jejunum to distal ileum, whereas transport was optimal in rat distal jejunum and proximal ileum and optimal in the distal jejunum in human BBMV (12). Regional-dependent studies were conducted here to determine possible differences in uptake of these drugs by sodium-dependent and sodium-independent systems in rabbit BBMV. Although IBG uptake was region-independent, jejunal uptake of GP was observed to be lower compared with duodenum and ileum at both temperatures and in the presence or absence of a sodium gradient. For GP, the data suggest that $b^{0,+}$ transport is weaker in rabbit jejunum than other regions of the small intestine and that this difference is evident even at the lower temperature for this amino acid exchanger. However, region-independent uptake is observed for IBG in the absence of sodium, a condition under which $b^{0,+}$ would be projected to dominate IBG uptake. One explanation for the fact that IBG transport is region independent regardless of temperature and medium sodium conditions, would be that entry of IBG into the vesicles by any pathway provides for stronger trans-stimulation of jejunal $b^{0,+}$ exchange uptake than is the case for GP.

Table I. Inhibition of 200 μM Pregabalin (IBG) Uptake in a Single Rabbit Small Intestinal BBMV Preparation

	Percent Inhibition	
Inhibitor	25 mM	50 mM
Pregabalin	38	50
L-leucine	35	36
Neurontin	31	35
L-phenylalanine	26	37
L-methionine	20	25
L-lysine	10	20
L-arginine	0	25
L-proline	7	2

Note. L-proline, L-glycine, β-alanine, γ-amino butyric acid (GABA), glycyl L-proline and cephalexin did not inhibit pregabalin uptake.

Species differences in the distribution and regulation of amino acid transport activity in the small intestine may limit implications of this animal data for human absorption of the gabapentenoids. For example, a recent study reports that leucine transport in human small intestinal BBMV is primarily mediated by B^0, whereas in rabbit, leucine transport also contains a small $b^{0,+}$ component (13). However, the intestinal transport differences between these structurally similar compounds are intriguing. Given that these compounds are not metabolized or protein bound in most species (6), the data provided here suggest that differences in transport of the two gabapentenoids by amino acid carriers could provide differences in pharmacokinetic profiles for oral absorption, organ distribution, and renal elimination. Individual absorption differences for IBG could be of particular importance because $B^{0,+}$ activity has recently been suggested to be a strong function of diet (5,14).

ACKNOWLEDGMENTS

This investigation was supported in part by Pfizer Global Research & Development and in part by a University of Michigan College of Pharmacy Vahlteich Research Award.

REFERENCES

1. J. S. Bryans and D. J. Wustrow. 3-substituted GABA analogs with central nervous system activity. *Med. Res. Rev.* **19**:149–177 (1999).
2. M. S. Luer, C. Hamani, M. Dujovny, B. Gidal, M. Cwik, K. Deyo, and J. H. Fischer. Saturable transport of gabapentin at the blood-brain barrier. *Neurol. Res.* **21**:559–562 (1999).
3. B. H. Stewart, A. R. Kugler, P. R. Thompson, and H. N. Bockbrader. A saturable transport mechanism in the intestinal absorption of gabapentin is the underlying cause of the lack of proportionality between increasing dose and drug levels in plasma. *Pharm. Res.* **10**:276–281 (1993).
4. N. Jezyk, B. H. Stewart, X. Wu, and D. Fleisher. Transport of pregabalin in rat intestine and caco-2 monolayers. *Pharm. Res.* **16**:519–526 (1999).
5. T. Hatanaka, Y. Nabuchi, and H. Ushio. Transport of N(G)-nitro-L-arginine across intestinal brush border membranes by Na+-dependent and Na+-independent amino acid transporters. *Pharm. Res.* **16**:1770–1774 (1999).
6. K. O. Vollmer, A. von Hodenberg, and E. U. Kolle. Pharmacokinetics and metabolism of gabapentin in rat, dog and man. *Arz.-Forsc.* **36**:830–839 (1986).
7. H. Yuasa, G. L. Amidon, and D. Fleisher. Peptide carrier-mediated transport in intestinal brush border membrane vesicles of rats and rabbits: Cephradine uptake and inhibition. *Pharm. Res.* **10**:400–404 (1993).
8. B. H. Stewart, E. L. Reyner, and R. H. Lu. Gabapentin (Neurontin) transport across Caco-2 cell monolayers: Interactions with zwitterionic and cationic amino acid carriers. *Pharm. Res.* **11** (Suppl)(10):S–254 (1994).
9. V. Ganapathy, M. E. Ganapathy, and F. H. Leibach. Intestinal transport of peptides and amino acids. In K. E. Barret and M. Donowitz (eds.), *Gastrointestinal Transport: Molecular Physiology*, Academic Press, San Diego, CA, 2001 pp. 379–412.
10. G. Rossier, C. Meier, C. Bauch, V. Summa, B. Sordat, F. Verrey, and L. C. Kuhn. LAT2, a new basolateral 4F2hc/CD98-associated amino acid transporter of kidney and intestine. *J. Biol. Chem.* **274**:34948–54 (1999).
11. D. P. Rajan, R. Kekuda, W. Huang, L. D. Devoe, F. H. Leibach, P. D. Prasad, and V. Ganapathy. Cloning and functional characterization of a Na(+)-independent, broad-specific neutral amino acid transporter from mammalian intestine. *Biochim. Biophys. Acta* **1463**:6–14 (2000).
12. L. K. Munck and B. G. Munck. Variation in amino acid transport along the rabbit small intestine. Mutual jejunal carriers of leucine and lysine. *Biochim. Biophys. Acta* **1116**:83–90 (1992).
13. P. Iannoli, J. H. Miller, H. T. Wang, B. Bode, W. W. Souba, N. E. Avissar, and H. C. Sax. Characterization of L-leucine transport system in brush border membranes from human and rabbit small intestine. *Metab Clin. Exper.* **48**:1432–1436 (1999).
14. T. Hatanaka, Y. Nabuchi, and H. Ushio. Na(+)-dependent and Na(+)-independent transport of L-arginine and L-alanine across dog intestinal brush border membrane vesicles. *Comp. Biochem. Physiol. Part B, Biochem. Mol. Biol.* **123**:105–13 (1999).