Transport of Pregabalin in Rat Intestine and Caco-2 Monolayers

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Purpose. The purpose of this study was to determine if the intestinal transport of pregabalin (isobutyl γ-aminobutyric acid, isobutyl GABA), a new anticonvulsant drug, was mediated by amino acid carriers with affinity for large neutral amino acids (LNAA).

Methods. Pregabalin transport was studied in rat intestine and Caco-2 monolayers. An *in vitro* Ussing/diffusion chamber model and an *in situ* single-pass perfusion model were used to study rat intestinal transport. An *in vitro* diffusion chamber model was used to evaluate Caco-2 transport.

Results. In rat ileum, pregabalin transport was saturable and inhibited by substrates of intestinal LNAA carriers including neurontin (gabapentin), phenylalanine, and proline. Weak substrates of intestinal LNAA carriers (β-alanine, γ-aminobutyric acid, and methyl aminoisobutyric acid) did not significantly change pregabalin transport. In Caco-2 monolayers that showed a high capacity for phenylalanine transport, pregabalin transport was concentration- and direction-independent and equivalent in magnitude to the paracellular marker, mannitol. The *in vitro* and *in situ* rat ileal permeabilities of the LNAA carrier-mediated compounds neurontin, pregabalin, and phenylalanine correlated well with the corresponding *in vivo* human oral absorption.

Conclusions. The transport of pregabalin was mediated by LNAA carriers in rat ileum but not in Caco-2 monolayers. Caco-2 was not an appropriate model for evaluating the *in vivo* human oral absorption of pregabalin and neurontin.

KEY WORDS: Caco-2; carrier-mediated transport; rat intestine; large neutral amino acid (LNAA); intestinal absorption models.

INTRODUCTION

The antiepileptic drug gabapentin, neurontin®, (Fig. 1, Table I) was designed as an agonist of the inhibitory neurotransmitter γ-aminobutyric acid (GABA) (1). Low energy conformations of neurontin are similar to those of the α-amino acid L-leucine (1). In rat intestinal epithelial cells, rat brain synaptosomes, and rat astrocytes, transport of neurontin was inhibited by the large, neutral amino acids (LNAA) L-phenylalanine and L-leucine suggesting that gabapentin utilizes one or more of the LNAA-type carriers (1–3). Stewart and colleagues (2) proposed that saturation of carrier-mediated intestinal transport was responsible for the dose-dependent extent of neurontin absorption observed in humans.

Development of second generation GABA derivatives identified pregabalin as a potent and effective antiepileptic drug (4). Pregabalin is a γ-amino acid similar to neurontin with respect to structure and physicochemical properties (Fig. 1, Table I). The two compounds share a unique binding site in rat brain (4). It was expected that pregabalin and neurontin utilize similar transport pathways in their intestinal absorption. Based on previous studies with neurontin, it was anticipated that pregabalin intestinal transport would be mediated by a LNAA carrier system with affinity for phenylalanine.

The first objective of this study was to characterize the intestinal transport of pregabalin. An emphasis was placed on identifying carrier-mediated transport by a system with an affinity for phenylalanine. In the absence of carrier-mediated transport, the absorption of this small, hydrophilic compound was projected to be predominately paracellular. An *in vitro* Ussing/diffusion chamber system was used to study transport in rat intestine and Caco-2 monolayers. Rat intestinal transport was also studied with an *in situ* single-pass perfusion model. In addition to comparing pregabalin and neurontin transport, phenylalanine was used as a LNAA transport reference and mannitol was used as a paracellular transport reference. The inhibition profile and concentration-dependence of pregabalin transport in rat ileum were consistent with carrier-mediated transport by a LNAA carrier system with an affinity for phenylalanine. As a result, pregabalin, neurontin, and phenylalanine provided a compound series with which to evaluate LNAA carrier-mediated drug transport in *in vitro* and *in situ* intestinal absorption models.

The second objective of this study was to assess the capacity of these *in vitro* and *in situ* models to be predictive of the clinical absorption data for neurontin and pregabalin. Intestinal permeability data from a number of *in vitro/in situ* experimental systems have been utilized to develop correlations with *in vivo* human absorption for compounds with a broad range of properties (5–11). In some cases, solutes absorbed by carrier-mediated processes did not fit into established correlations (5–7). Since the extent of carrier-mediated transport of pregabalin and neurontin differed among the experimental systems utilized in this study, the potential for these systems to project the extent of human absorption in clinical studies was also evaluated.

MATERIALS AND METHODS

Chemicals

Pregabalin (isobutyl GABA), neurontin (gabapentin), ^{14}C-isobutyl GABA (5.03 mCi/mmole), and ^{14}C-gabapentin (4.26 mCi/mmole) were donated by Parke-Davis/Warner-Lambert (Ann Arbor, MI). Additional radiolabeled compounds included ^{3}H-L-phenylalanine (43 Ci/mmole) from Amersham (Arlington Heights, IL), ^{3}H-mannitol (30 Ci/mmole), ^{3}H-PEG 4000 (1.87 mCi/g), and ^{14}C-PEG 4000 (specific activity unknown) from NEN/Dupont (Boston, MA). All other chemicals were obtained from Sigma (St. Louis, MO) and were used as received. Only L-amino acids were used. Ketamine-HCl, from Fort Dodge Lab (Fort Dodge, IA), and Rompum (xylazine), from Bayer (Shawnee Mission, KS), were used as anesthetics. The scintillation cocktail Ecolite^{+} was produced by ICN (Costa Mesa, CA).

Animals

Pathogen-free, male, Sprague-Dawley rats were obtained from Charles River Laboratories. Rats weighed from 350 g to

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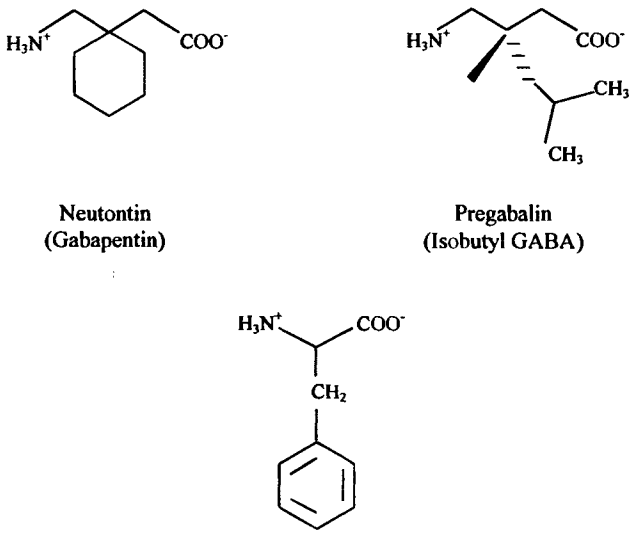

Fig. 1. The structures of the antiepileptic compounds neurontin (gabapentin) and pregabalin (isobutyl GABA) and the amino acid phenylalanine.

450 g and were fasted overnight prior to an experiment. An IM injection of 100 mg/kg ketamine and 20 mg/kg xylazine was used to induce anesthesia. Protocols followed the "Principles of Laboratory Animal Care" (NIH publication #85-23, revised 1985).

In Vitro Intestinal Diffusion Chamber Studies

Procedures for the intestinal diffusion chamber studies were based on the methods reported by Grass and Sweetana (12). Small sections of excised rat intestine, 2.5 cm to 3 cm in length, were opened along the mesenteric border then secured in a side-by-side diffusion chamber. The external muscle layers were stripped from colonic tissue. Small intestinal tissue was used unstripped. Assembled diffusion chambers were placed in a 37°C heating block, connected to a 95% O_2/5% CO_2 air-lift, and filled with 4 or 5 ml of 37°C buffer. All intestinal diffusion chamber transport studies were conducted in the mucosal (donor)-to-serosal (receiver) direction. Samples of the buffer in the donor (0.03 ml to 0.05 ml) and receiver (1 ml) chambers were obtained at appropriate time points (12). The diffusion chambers and the air-lift/heating block assembly were purchased from Precision Instrument Design (Lake Tahoe, CA) and Costar (Cambridge, MA), respectively.

The buffer used throughout the diffusion chamber experiments contained 112 mM NaCl, 5 mM KCl, 1.2 mM $CaCl_2$, 1.2 mM $MgCl_2$, 25 mM $NaHCO_3$, 1.6 mM Na_2HPO_4, 0.4 mM NaH_2PO4, 5 mM glucose, and 5 mM mannitol. When perfused with 95% O_2/5% CO_2 and maintained at 37°C, the buffer pH was 7.4. Buffer osmolality was 290 ± 15 mmol/kg (Wescor Vaporpressure Osmometer, Wescor Company, Logan, UT).

The steady-state flux (J_{ss}) and effective permeability (P_{eff}) were based on the appearance of compound in the receiver buffer under sink conditions

$$P_{eff} = \frac{J_{ss}}{C_D} = \left(\frac{dC_R \cdot V_R}{dt \cdot A}\right)\left(\frac{1}{C_D}\right)$$

where dC_R/dt is the steady-state change in compound concentration in the receiver buffer over time, V_R is the volume of the receiver buffer, A is the exposed surface area of the tissue (1.36 cm^2), and C_D is the compound concentration in the donor chamber.

Inhibition studies were designed so each tissue served as its own control. A P_{eff} value was determined for a 20 minute period before (control) and after (inhibition) adding 0.05 ml to 1 ml of inhibitor to one side of each diffusion chamber. If necessary, mannitol was used to adjust the osmolality of the opposite chamber and/or additional radiolabeled drug was added to the donor chamber to maintain constant donor drug concentration. Data was recorded as an inhibition ratio: $P_{eff\ inhibitor}/P_{eff\ control}$.

Table I. The Physicochemical Properties of the Antiepileptic GABA Derivatives Neurontin and Pregabalin, the Paracellular Reference Transport Reference Compound Mannitol, and the Amino Acid Carrier-Mediated Transport Reference Phenylalanine

Compound	Molecular weight	Aqueous solubility (mg/ml)[a]	Log D (octanol/water)	pKa
Neurontin	171.24	>100[b]	−1.10[b]	3.7, 10.7
Pregabalin	159.23	32.1[b]	−1.35[b]	4.0, NA[d]
Phenylalanine	165.19	29.6[c]	−1.40[c]	1.8, 9.1
Mannitol	182.17	182.0[c]	−3.10[c]	13.5

Note: Information for neurontin obtained from reference 26, for pregabalin from reference 14, for phenylalanine from references 27, 28, 29, for mannitol from reference 29 and 30.
[a] Aqueous solubilities determined at room temperature.
[b] pH 7.4.
[c] pH not reported.
[d] Not available.

In Situ Intestinal Perfusion Studies

Procedures for the perfusion studies were based on the methods reported by Lu *et al.* (13) and Stewart *et al.* (2). Small incisions were made at both ends of an approximately 14 cm long segment of the exposed rat small intestine. A cannula was inserted at each end of the segment and secured with suture thread. The inlet tubing was connected to an infusion pump (Harvard Apparatus Company, South Natick, MA) and the outlet tubing rested on the rat's abdomen. Tubing between the infusion pump and the intestine was water-jacketed and maintained at 37°C. A heat lamp was used to warm the rats.

The perfusion buffer contained 10 mM MES, 135 mM NaCl, 5 mM KCl, and 0.01% PEG 4000 (w/v). The pH was adjusted to 6.5 with NaOH. PEG 4000, traced with radiolabeled PEG 4000, served as a water-transport marker. Inhibitors were added to the perfusion buffers without correcting for osmolality. The presence of 20 mM inhibitor did not change the perfusion buffer pH.

The effective permeability (P_{eff}) measured by intestinal perfusion was based on the loss of drug from the perfusate

$$P_{eff} = \left(-\frac{Q}{2\pi rL}\right)\left(\ln \frac{C'_{out}}{C_{in}}\right)$$

where Q is the perfusate flow rate through the segment (0.123 ml/min), r is the radius of the segment (0.18 cm), L is the length of the perfused segment, and C_{in} is the drug concentration of the perfusate entering the intestinal segment. C'_{out} is the drug concentration in the exiting perfusate, C_{out}, corrected for water transport according to the change in the concentration of the non-absorbable compound PEG 4000

$$C'_{out} = \frac{C_{out}}{(PEG_{out}/PEG_{in})}$$

The length of the perfused segment was measured by laying a thread along the segment *in situ*.

Caco-2 Cell Culture and Transport Studies

The Caco-2 cell line, passage 17, was obtained from American Type Culture Collection (Rockville, MD). Cells were grown in 75 cm^2 or 162 cm^2 culture flasks and maintained at 37°C in a humidified atmosphere of 5% CO_2. The culture media consisted of Dulbecco's Modified Eagle's Medium (Sigma, St. Louis, MO), pH 7.4, containing 25 mM D-glucose, 25 mM HEPES, 44 mM $NaHCO_3$, and 4 mM L-glutamine, supplemented with 10% (v/v) fetal bovine serum (Gibco, Grand Island, NY; Sigma, St. Louis, MO) and 1% (v/v) non-essential amino acids (Sigma, St. Louis, MO). Culture media was changed every two days. Cultures were passaged, 1:5, approximately every five days using 0.25% trypsin containing 0.2% EDTA. For transport studies, cells were seeded onto 1.13 cm^2 polycarbonate Snapwell™ (Costar, Cambridge, MA) membranes (without collagen coating) at a density of 63,000 cells/cm^2. Media in the Snapwells™ was changed every two days for the first ten days following seeding then replaced every day thereafter. Caco-2 monolayers used for transport studies were of passage 37 to 47 and grown for 19 to 30 days on the filters. These conditions had been previously verified to be optimal for phenylalanine transport.

Caco-2 transport studies were performed using a side-by-side diffusion chamber system (Costar, Cambridge, MA). The procedure is similar to the previously described intestinal diffusion chamber study procedure. The volume of each half-chamber was five milliliters. Caco-2 transport studies were performed in the mucosal-to-serosal and serosal-to-mucosal directions. The buffer used for the Caco-2 transport studies contained 130.8 mM NaCl, 5.55 mM KCl, 1.25 mM $CaCl_2$, 1.10 mM $MgCl_2$, 10 mM HEPES, 1.65 mM Na_2HPO_4, 0.3 mM HaH_2PO_4 and either 10 mM mannitol (mucosal buffer) or 10 mM glucose (serosal buffer). Buffer maintained at 37°C and aerated with 95% O_2/5% CO_2 had a pH of 6.5.

The effective permeability across Caco-2 monolayers was determined from the measured steady-state flux across the monolayer and was calculated as described for permeability across excised rat intestine mounted in diffusion chambers.

Compound Analysis

Pregabalin and neurontin are not metabolized by the rat or human (L. L. Radulovic, Parke-Davis personal communication, 14) therefore radioactivity was an appropriate measure of each drug. All samples from the intestinal diffusion chamber, intestinal perfusion, and Caco-2 diffusion chamber studies were mixed with 5 ml of scintillation cocktail. Sample radioactivity was measured with a Beckman LS 5000 TD liquid scintillation counter using a dual label or single label counting program, as appropriate, and an external standardization quench method.

Data Presentation and Statistical Analysis

All data is presented as mean ± standard error of the mean (sem). Unless noted, a one-factor ANOVA, at a 95% significance level, was used to analyze the data. Fisher's Protected Least Significant Difference (PLSD) was used to make pairwise comparisons. Statistics were calculated by StatView SE+Graphics (version 1.03).

RESULTS AND DISCUSSION

Reference Compounds: Passive Transport in Rat Colon

Transport of phenylalanine and mannitol served as reference of LNAA carrier-mediated and paracellular transport, respectively, in each model. Phenylalanine transport was saturable and/or inhibited by a LNAA in each model indicating that rat ileum and Caco-2 monolayers maintained LNAA carrier-mediated transport under the described experimental conditions.

Significant carrier-mediated transport of phenylalanine was not expected to occur in rat colon. Previous Ussing chamber studies indicated a lack of carrier mediated amino acid transport in mammalian colon (15). Though carrier-mediated transport may occur in the colon, it appears to provide a negligible contribution to total intestinal absorption. Consequently, rat colon was used to study the passive transport of pregabalin. The effective permeabilities of pregabalin ($P_{eff\,IBG}$) and mannitol ($P_{eff\,MAN}$) were determined simultaneously in each of six colonic tissue samples. The linear regression function between $P_{eff\,IBG}$ and $P_{eff\,MAN}$ ($y = 1.01x - 0.44 \times 10^{-6}$, $r^2 = 0.98$) had a slope not significantly different from one and a y-intercept not significantly different from zero (one-group t-test). There was a similar relationship between the effective permeability of neurontin ($P_{eff\,GBP}$) and $P_{eff\,MAN}$ ($y = 0.94x - 0.12 \times 10^{-6}$, $r^2 = 0.96$, $n = 6$). These correlations suggest that the passive transport of pregabalin and neurontin were significantly paracellular and equal to the transport of mannitol (16,17). As a result, the value of $P_{eff\,MAN}$ served as an estimate of the passive permeabilities of pregabalin and neurontin.

Studies in Rat Small Intestine

Rat Ileal Permeability

The P_{eff}s of phenylalanine, the carrier-mediated transport reference, and mannitol, the passive transport reference, established the high and low values for the range of P_{eff}s determined with the *in vitro* diffusion chamber and *in situ* perfusion systems. The *in vitro* rat ileal P_{eff} of pregabalin may be compared to the *in vitro* ileal P_{eff} of neurontin, phenylalanine, and mannitol in Fig. 2. $P_{eff\,IBG}$ was significantly greater than $P_{eff\,MAN}$ and $P_{eff\,GBP}$. The characteristics of *in situ* rat small intestinal perfusion permeability were similar to the characteristics of *in vitro* rat small intestinal diffusion chamber permeability (Fig. 2). The rank order of the perfusion ileal P_{eff}s was $P_{eff\,PHE} > P_{eff\,IBG} >$

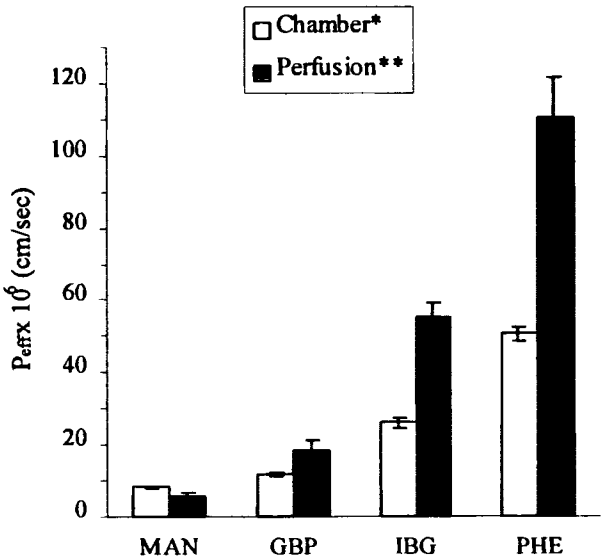

Fig. 2. The effective permeability (P_{eff}) of 5 mM mannitol (MAN), 0.01 mM neurontin (GBP), 0.01 mM pregabalin (IBG), and 0.01 mM phenylalanine (PHE) in rat ileum determined with the *in vitro* diffusion chamber and *in situ* single-pass perfusion models of intestinal absorption. Data represent mean ± sem of at least four measurements. * Significant difference between diffusion chamber P_{eff}: $P_{eff\ PHE} > P_{eff\ IBG} > P_{eff\ GBP} = P_{eff\ MAN}$. ** Significant difference between P_{eff}: $P_{eff\ PHE} > P_{eff\ IBG} > P_{eff\ GBP} > P_{eff\ MAN}$. A one-factor ANOVA test (95% confidence level) and Fisher's Protected Least Significant Difference (PLSD) were used to compare the data.

$P_{eff\ GBP} > P_{eff\ MAN}$. A clear concentration dependence of mucosal-to-serosal pregabalin permeability across rat ileum in the diffusion chamber (Fig. 3) provides some evidence for carrier-mediated transport in this experimental system.

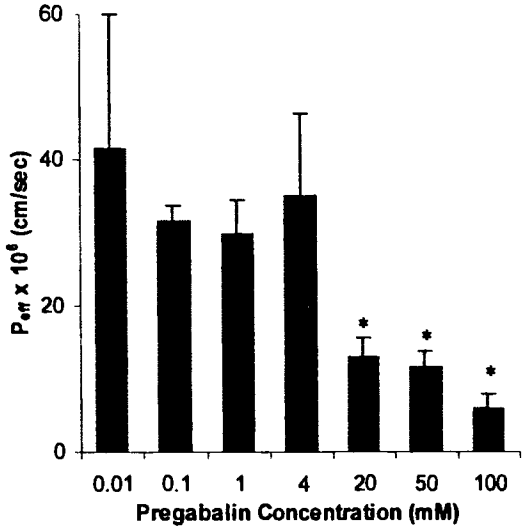

Fig. 3. Mucosal-to-serosal effective permeability as a function of pregabalin concentration in rat ileum determined with the *in vitro* diffusion chamber. Data represent mean ± SD from three rats with duplicate measurements (n = 6). * Significantly decreased as compared to permeability at 0.10 mM (t-test, $p < 0.05$).

Inhibition Studies

The group of amino acids tested as inhibitors of the *in vitro* ileal transport of pregabalin included strong and weak substrates of a LNAA carrier system. Phenylalanine and neurontin are strong substrates of the LNAA carrier system in rat intestine (2). Evidence that phenylalanine and neurontin were substrates of a LNAA carrier system in the *in vitro* rat ileum-diffusion chamber preparations included inhibition by the LNAA leucine, saturable transport, and cross-inhibition (Table II). The α-amino acid proline significantly inhibited phenylalanine transport as well (Table II). Proline and phenylalanine have been characterized as substrates of the same neutral α-amino acid carrier system (system B) (18). Amino acids that are weak substrates for LNAA carrier systems, β-alanine (19), GABA (19), and methyl aminoisobutyric acid (MeAIB) (18), did not significantly change $P_{eff\ PHE}$ (Table II).

Pregabalin and phenylalanine were expected to share a carrier system. Cross-inhibition suggested that there was a common carrier system for pregabalin and phenylalanine transport. The inhibition of one amino acid's transport by another does not confirm a shared carrier for the two compounds (20). However, two additional findings were also consistent with a common carrier system for pregabalin and phenylalanine in rat ileum. First, the inhibition profiles of pregabalin and phenylalanine transport were similar (Table II). Second, when measured in the same tissue samples *in vitro*, $P_{eff\ IBG}$ was linearly correlated to $P_{eff\ PHE}$ ($y = 0.62x - 2.43 \times 10^{-6}$, $r^2 = 0.91$, $n = 73$).

The effect of inhibitors on *in situ* intestinal transport was similar to the described effects on *in vitro* intestinal transport. The *in situ* ileal transport of pregabalin and phenylalanine was saturable, decreasing 54% and 37%, respectively, at 20 mM relative to 0.01 mM (Table III). 20 mM neurontin significantly decreased the *in situ* ileal permeability of 0.01 mM pregabalin and 0.01 mM phenylalanine by 68% and 18%, respectively. The *in situ* ileal P_{eff} of 0.01 mM phenylalanine was decreased 30% by 20 mM pregabalin.

Pregabalin versus Neurontin Transport

The structural and physicochemical similarity between pregabalin and neurontin suggested that the transport of pregabalin would be similar to the transport of neurontin. The observed similarities between the *in vitro* transport of pregabalin and neurontin in the rat intestine-diffusion chamber model were: 1) mediated transport in the small intestine by a carrier of phenylalanine and 2) equal passive permeability in the colon. Though both pregabalin and neurontin appeared to share a carrier with phenylalanine, it is not clear that pregabalin and neurontin share a carrier. At 20 mM inhibitor, there was no cross-inhibition between pregabalin and neurontin. Self-inhibition of neurontin transport was demonstrated between 0.01 mM and 50 mM but not between 0.01 mM and 20 mM. A concentration of pregabalin greater that 20 mM may be needed to significantly inhibit neurontin transport in the *in vitro* rat ileum-diffusion chamber model.

The characteristics of pregabalin and neurontin transport in the rat ileum were similar, however the magnitude of transport was different. In the *in vitro* diffusion chamber and *in situ* perfusion models, the ileal P_{eff} of pregabalin was greater than the ileal P_{eff} of neurontin. The passive permeabilities of pregabalin and neurontin were considered equal and therefore the

Table II. The Effect of 10 mM Inhibitors on the *In Vitro* Effective Permeability (P_{eff}) of 0.01 mM Neurontin, 0.01 mM Pregabalin, and 0.01 mM Phenylalanine in Unstripped Rat Ileum Measured with the Diffusion Chamber Model

	Inhibition Ratio[a]					
Inhibitor[b]	Neurontin	n[c]	Pregabalin	n	Phenylalanine	n
Control[d]	1.08 ± 0.04	7/3	0.90 ± 0.02	15/7	0.85 ± 0.02	16/7
Pregabalin	1.16 ± 0.14	5/3	0.57 ± 0.05[e]	9/3	0.70 ± 0.05[e]	13/6
Neurontin	1.24 ± 0.06	3/2	0.58 ± 0.09[e]	5/3	0.69 ± 0.04[e]	8/5
	0.86 ± 0.08[e]	7/3	ND[d]	—	0.60 ± 0.06[e]	7/3
Leucine	0.82 ± 0.09[e]	6/2	ND	—	0.43 ± 0.10[e]	6/2
Phenylalanine	0.71 ± 0.08[e]	6/2	0.50 ± 0.06[e]	8/2	0.53 ± 0.03[e]	14/4
Proline	ND	—	0.66 ± 0.03[e]	7/3	0.70 ± 0.02[e]	7/3
Glycine	ND	—	0.67 ± 0.04[e]	4/1	0.72 ± 0.02	4/1
-Alanine	ND	—	0.90 ± 0.05	5/2	0.89 ± 0.04	5/2
GABA	ND	—	0.93 ± 0.03	8/4	0.95 ± 0.03	8/4
MeAIB	ND	—	0.91 ± 0.03	6/2	0.92 ± 0.06	6/2

[a] Inhibition Ratio = $P_{eff\ inhibitor}/P_{eff\ control}$. Each tissue sample served as its own control. The $P_{eff\ control}$ was determined from minutes 20 to 40 of the experiment, inhibitor was added to each diffusion chamber at minute 43, $P_{eff\ inhibitor}$ was determined from minutes 60 to 80 of the experiment. Data represent mean ± sem. Values less than one indicate decreased permeability, values greater than one indicate enhanced permeability.
[b] Control measurements determined the effect of time on P_{eff}. No inhibitor was added during the control studies.
[c] Number of tissue samples/number of rats.
[d] Not determined.
[e] Significantly different from control (ANOVA, 95% confidence level, Fisher's PLSD comparison test).

difference between the effective permeabilities of pregabalin and neurontin in the rat ileum was likely a result of greater mucosal-to-serosal carrier-mediated permeability for pregabalin. Greater carrier-mediated permeability for one compound compared to another may be due to better affinity for a common carrier system or a greater number of carrier systems for one compound (21). A similar argument can be made for the greater effective permeability of phenylalanine compared to neurontin and pregabalin. This is based on the assumption that the passive permeability of phenylalanine was equal to the passive permeabilities of neurontin and pregabalin. This is a reasonable assumption because the molecular weights, partition coefficients, and structures of all three compounds are similar. The P_{eff} of mannitol is regarded as an estimate of the passive P_{eff} of neurontin and pregabalin. In some *in vitro* diffusion chamber studies, the P_{eff} of mannitol was determined simultaneously with the P_{eff} of pregabalin, neurontin, or phenylalanine. Therefore, the relative permeability, or ratio $P_{eff\ n}/P_{eff\ m}$ where n represents neurontin, pregabalin, or phenylalanine and m represents mannitol, may be used to compare the magnitude of carrier-mediated transport of neurontin, pregabalin, and phenylalanine. The relative permeability of neurontin, pregabalin, and phenylalanine were 41%, 91%, and 103%, respectively, greater in the rat ileum than in the rat jejunum. Region-dependent phenylalanine transport by a single carrier in mammalian small intestine has been attributed to differences in carrier density, not affinity, by other researchers (22). As reported for transport comparisons of other amino acids in small intestine, the similar percentage change in the P_{eff} of pregabalin and phenylalanine may be indicative of more common carriers between pregabalin and phenylalanine than between neurontin and phenylalanine (21).

Table III. The Effective Permeabilities (P_{eff}) 5 mM Mannitol, 0.01 mM Neurontin, 0.01 mM Pregabalin, and 0.01 mM Phenylalanine Measured in the *In Situ* Single-Pass Perfused Rat Small Intestine

Study		$P_{eff} \times 10^6$ (cm/sec)[a]	
Compound	Concentration or Inhibitor	Ileum	n[b]
Mannitol	5 mM	5.54 ± 1.04	4
Neurontin	0.01 mM	18.60 ± 2.75	7
	+20 mM Pregabalin	7.57 ± 1.27	5
Pregabalin	0.01 mM	55.42 ± 4.00	6
	20 mM	25.29 ± 4.61[c]	4
	+0.01 mM Neurontin	17.56 ± 2.81[c]	4
Phenylalanine	0.01 mM	110.50 ± 11.17	4
	20 mM	69.48 ± 6.06[c]	4
	+0.01 mM Neurontin	91.01 ± 8.82[c]	4
	+0.01 mM Pregabalin	77.48 ± 6.30[c]	5

[a] Data represent mean ± sem.
[b] Number of measurements.
[c] Significantly different from 0.01 mM (ANOVA, $p < 0.05$; Fisher's PLSD).

Caco-2 Monolayers

The Caco-2 studies compared the transport of pregabalin to the transport of neurontin, the paracellular transport reference mannitol, and the carrier-mediated transport reference phenylalanine (Table IV). The value of $P_{eff\ GBP}$ is similar to the value of $P_{eff\ GBP}$ reported by Stewart and colleagues (9). The magnitude of pregabalin P_{eff} in Caco-2 monolayers was on the order of the paracellular reference compound P_{eff}. In contrast, the magnitude of pregabalin P_{eff} in rat ileum was on the order of the magnitude of the carrier-mediated reference compound P_{eff}.

There was no evidence of carrier-mediated transport of pregabalin in Caco-2 monolayers. Again, this is in contrast to carrier-mediated pregabalin transport in rat ileum. In Caco-2 monolayers, the P_{eff} of pregabalin was determined for several donor pregabalin concentrations (Fig. 4). Within the range 0.01

Table IV. The Effective Permeabilities (P_{eff}) of 10 mM Mannitol, 0.01 mM Neurontin, 0.01 mM Pregabalin, and 0.01 mM Phenylalanine Across Caco-2 Monolayers in the Mucosal-to-Serosal Direction

Compound	Passage	$P_{eff} \times 10^6$ (cm/sec)[a]	n[b]
Mannitol	37	1.31 ± 0.34	5
	42	0.96 ± 0.13	8
Neurontin	37	2.74 ± 0.17	3
Pregabalin	47	1.18 ± 0.07	3
Phenylalanine	37	42.78 ± 3.32	5
	42	62.76 ± 2.24[c]	8

[a] Data represent mean ± sem.
[b] Number of measurements.
[c] Statistically greater than passage 37 (two-way t-test, $p < 0.05$).

mM to 50 mM, pregabalin P_{eff} in Caco-2 monolayers was concentration-and direction-independent. The apparent lack of pregabalin carrier-mediated transport in Caco-2 monolayers occurred despite a clear demonstration of phenylalanine carrier-mediated transport. The P_{eff} of 0.01 mM phenylalanine was significantly decreased 58% by 1 mM phenylalanine (passage 37) and 48% by 1 mM alanine (passage 42).

Pregabalin transport in the apical-to-basolateral direction was also studied as a function of concentration from 0.01 to 100 mM (passage 24) in the laboratories of Parke-Davis/Warner-Lambert. While 100 μM pregabalin and mannitol permeabilities were only 50% of those values reported from the laboratory at the University of Michigan, pregabalin permeabilities were also independent of concentration and equivalent to mannitol permeability. Furthermore, when calcium was removed from the apical medium, both mannitol and pregabalin permeability increased 6-fold suggesting pregabalin transport in Caco-2 cells is predominantly paracellular. In both Caco-2 systems, phenylalanine transport was optimum and shown to be carrier-mediated (21 days in culture).

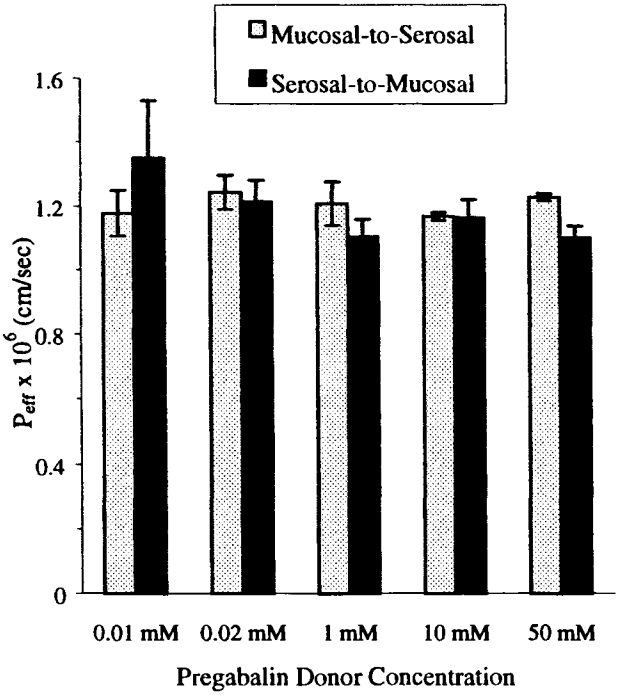

Fig. 4. The mucosal-to-serosal and serosal-to-mucosal effective permeability (P_{eff}) of pregabalin across Caco-2 monolayers. Monolayers were of passage 44 to 47 and used 22 days post-seeding. Data represent mean ± sem of three measurements. There was no significant directional- or concentration-dependence to $P_{eff\ IBG}$ (one-factor ANOVA, $p > 0.05$).

Permeability in Experimental Systems and Human Fraction Dose Absorbed

The transport of the carrier-mediated compounds neurontin, pregabalin, and phenylalanine was qualitatively similar in the *in vitro* rat ileum-diffusion chamber model and the *in situ* rat ileum perfusion model. Furthermore, the effective permeabilities of these carrier-mediated compounds measured in the two models were linearly correlated (diffusion chamber

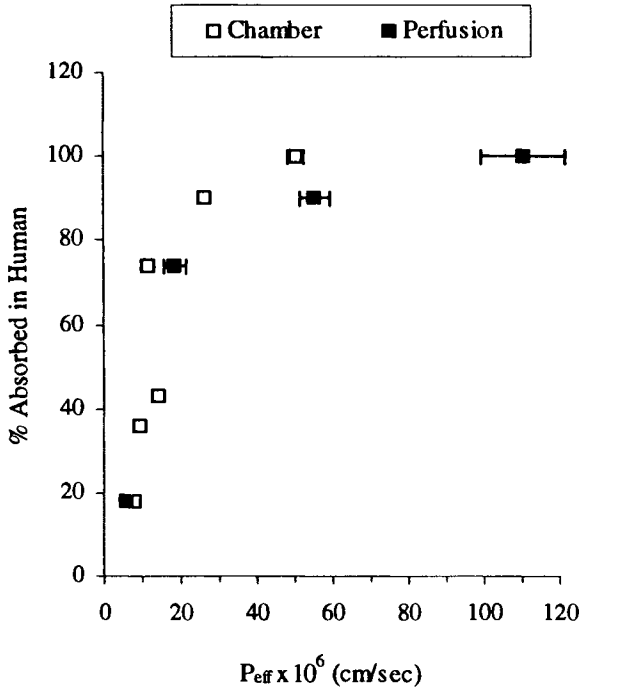

Fig. 5. The fraction (%) of oral dose absorbed by humans versus the effective permeability (P_{eff}) of rat ileum measured with an *in vitro* diffusion chamber and an *in situ* single-pass perfusion model of intestinal absorption. Data represent mean ± sem. Error bars are within symbol dimensions in some cases. The diffusion chamber P_{eff}s for 20 mM neurontin and 50 mM neurontin were estimated by multiplying the ileal P_{eff} of 0.01 mM gabapentin by the appropriate inhibition ratio (Table II). The human oral absorption data were from references 2, 9, 14, and 30.

Compound	% Absorbed
Mannitol	18
50 mM Neurontin	36
20 mM Neurontin	43
0.01 mM Neurontin	74
0.01 mM Pregabalin	90
0.01 mM Phenylalanine	100

versus perfusion: $y = 0.39x + 4.5 \times 10^{-6}$, $r^2 = 0.97$, $n = 5$). The P_{effs} measured in the *in situ* perfusion model were approximately 2.6 times greater than the P_{effs} measured in the *in vitro* diffusion chamber model. This factor is consistent with previous work comparing transport of carrier-mediated compounds in these two experimental systems (11,23). In both systems, the permeability of the paracellular transport reference, mannitol, corresponds to the lower limit, or expected passive permeability, of the P_{eff} of these carrier-mediated compounds.

Various correlations between *in vitro* and *in situ* drug permeabilities with fraction of drug absorbed in humans have been previously advanced in the literature (7–9). While there is no direct evidence that the absorption mechanism is the same in rat and human, rat ileal permeability of neurontin, pregabalin, and phenylalanine measured in either the diffusion chamber or intestinal perfusion system correspond well to human oral absorption. In this regard, the results of this study do show a correlation between the *in vitro/in situ* rat intestinal permeability and fraction of drug absorbed in humans (Fig. 5).

The P_{effs} of neurontin and pregabalin in Caco-2 monolayers were similar, therefore, it was not possible to identify pregabalin as the more extensively absorbed compound. In fact, while Caco-2 monolayers showed evidence of carrier-mediated phenylalanine transport as documented in earlier work (24), pregabalin and neurontin permeability are equivalent to mannitol permeability in this system and are not predictive of human absorption. Transport differences (sodium-dependence) between Caco-2 cells and mammalian small intestine have been previously reported for phenylalanine (24). The possibility that amino acid transport and metabolism differs between cancer cells and normal tissue may play a role in study observations (25).

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