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Research Paper

In Vivo Effects of Glycyl-Glutamate and Glycyl-Sarcosine on Gabapentin Oral Absorption in Rat

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Purpose. The objective of this study was to evaluate the *in vivo* consequences of glycyl-glutamate coadministration on gabapentin oral absorption.
Methods. Rats were administered gabapentin (10 mg/kg plus radiotracer) by gastric gavage, in the absence and presence of dipeptides, and by intravenous administration. Serial blood samples were obtained over 6 h and the pharmacokinetics of gabapentin were determined by noncompartmental analysis.
Results. Glycyl-glutamate coadministration increased the C_{max} of gabapentin by 86% as compared to gabapentin alone. In agreement, the oral absorption of gabapentin, relative to the intravenous dose, was 79% after glycyl-glutamate loading but only 47% when drug was administered alone. However, when glycyl-sarcosine was added to the orally administered admixture of gabapentin plus glycyl-glutamate, values for C_{max} and $AUC_{0-6\ h}$ reverted back to that of control. In contrast, the t_{max} and terminal half-life of gabapentin did not change after oral dosing for all treatments.
Conclusions. These findings are unique in demonstrating that under physiologic, *in vivo* conditions, the luminal presence of glycyl-glutamate could dramatically enhance the C_{max} and $AUC_{0-6\ h}$ of gabapentin. The results are consistent with previous *in situ* intestinal perfusion studies in rat, and establish a functional interaction between the activities of PEPT1 and amino acid exchangers.

KEY WORDS: absorption; gabapentin; GlyGlu; GlySar; trans-stimulation.

INTRODUCTION

Gabapentin is an amino acid-like drug derived from the inhibitory neurotransmitter γ-amino butyric acid (Fig. 1). It is a widely used antiepileptic agent with mechanism of action at the $\alpha_2\delta$ subunit of a voltage-dependent Ca^{2+} channel in the central nervous system (1–3). In addition to being zwitterionic at physiological pH, gabapentin has a small molecular weight of 171, low partition coefficient (log D = 1.1) and high water solubility (>100 mg/ml) (2). In both humans and rats, it is not systemically metabolized and has very minimal or no protein binding (2). Gabapentin oral absorption is dose-dependent suggesting the presence of carrier saturation (4) and permeation across the blood-brain barrier is facilitated by the large neutral amino acid transporter, system LAT1 (5,6). Previously, we evaluated the effective permeability of gabapentin in the single-pass rat intestinal perfusion (RIP) model and found that its transport was independent of both proton and sodium and hindered by cationic amino acids (7). Accordingly, all the proton and sodium-dependent carriers at the intestinal epithelium were ruled out as candidates for gabapentin intestinal uptake. As a result, systems $b^{0,+}$ and LAT2 at the apical and basolateral membranes, respectively, were believed to be the carriers responsible for the directional uptake of gabapentin from the intestinal lumen into the system circulation.

An interaction of gabapentin with apical $b^{0,+}$ was confirmed by the significant inhibition of the drug permeability in the presence of the high affinity, basic amino acids substrates of $b^{0,+}$ (i.e. lysine and arginine) (7). These findings were also validated by other investigators using various intestinal models, in which gabapentin interfered with the uptake of neutral and cationic amino acid substrates of systems $b^{0,+}$ and LAT2 (4), and vice versa (8–10). System $b^{0,+}$, a proton- and sodium-independent transporter of small neutral and cationic amino acids, can be found at the intestinal brush border membrane (11). System LAT2 is also a proton- and sodium-independent carrier, but it is localized to the basolateral membrane and only facilitates the transport of large neutral amino acids (12). Both of these carriers belong to the solute carrier families 3 and 7 (SLC3 and SLC7) (11–13), and are known as exchangers. Thus, the uptake of an extracellular substrate is linked to the efflux of an intracellular substrate.

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ABBREVIATIONS: $AUC_{0-6\ h}$, area under the blood concentration vs. time curve from 0–6 h; C_{max}, maximal blood concentration; GB, gabapentin; GlyGlu, glycyl-glutamate; GlySar, glycyl-sarcosine; IV, intravenous dosing; RIP, rat intestinal perfusion; SLC, solute carrier family; $T_{1/2,\lambda z}$, terminal half-life of drug; t_{max}, time of maximal blood concentration.

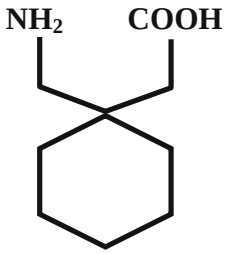

Fig. 1. Chemical structure of gabapentin.

Therefore, through cellular loading, it is possible to affect the uptake activity of these carriers by influencing the intracellular concentration of exchangeable substrates.

Wenzel *et. al.* (14) demonstrated previously that dipeptide loading in Caco-2 cells was capable of trans-stimulating amino acid uptake across the $b^{0,+}$ exchanger. They further demonstrated a functional relationship between PEPT1-mediated uptake of dipeptides, hydrolysis to constituent amino acids, and the enhanced absorption of amino acids in a cellular model. We have extended these findings by demonstrating, for the first time, that the same interaction can be established in an *in situ* RIP model for an amino acid-like drug (7). Our studies incorporated the loading of the intestinal epithelium with glycyl-glutamate (GlyGlu) through PEPT1 in order to enhance gabapentin uptake through exchanger $b^{0,+}$. With this strategy we were able to achieve a significant improvement in the drug effective permeability versus the control. Through inhibition studies, we were able to determine that the loaded GlyGlu underwent hydrolysis to produced free amino acids that were subsequently used to trans-stimulate drug exchange. However, results in a RIP model may not necessarily correspond to what really transpires in an *in vivo* setting. Therefore, our goal was to evaluate whether a functional relationship exists *in vivo* between PEPT1 activity and the enhanced absorption of gabapentin by $b^{0,+}$ exchange.

MATERIALS AND METHODS

Chemicals

The dosing buffer, consistent with the uptake buffer used in the RIP studies, contained 10 mM MES/Tris, 135 mM NaCl, 5 mM KCl and 0.01% (w/v) PEG 4000, with pH adjusted to 7.4 by modifying the MES/Tris ratio. All chemicals used in the buffer were purchased from Sigma-Aldrich (St. Louis, MO). Unlabeled and [^{14}C]-labeled gabapentin were obtained from Sigma-Aldrich (St. Louis, MO) and Perkin Elmer (Wellesley, MA), respectively. The dipeptides [glycyl-glutamate (GlyGlu) and glycyl-sarcosine (GlySar)] were purchased from Bachem (Torrance, CA).

Trans-stimulation Studies

Male Sprague–Dawley rats (280–360 g) were acquired from Charles River Laboratory (Wilmington, MA) and fasted overnight (12–18 h, water ad libitum) prior to each experiment. As a control, the rats were given, by gastric gavage, a 2 ml buffer solution containing only drug (10 mg/kg of unlabeled gabapentin plus 2.1 μCi of [^{14}C]-gabapentin; sp. act.=52 mCi/mmol). For the trans-stimulation studies, the animals received a predose of 1 ml GlyGlu (100 mM) solution by gastric gavage for cellular loading. One-half hour later, the predose was followed by a 2 ml mixture of gabapentin (10 mg/kg plus radiolabeled tracer) plus GlyGlu (100 mM).

Following oral dosing, the animals were sedated by intramuscular injection of a ketamine/xylazine cocktail (50 and 10 mg/kg, respectively), anesthetized with pentobarbital (40 mg/kg) by intraperitoneal injection, and placed in a heated chamber (at 31°C). Serial blood samples (100 μl) were then collected, via tail-vein nick, at 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4 and 6 h. The animals were kept hydrated throughout the experiment by intraperitoneal injections of 2 ml normal saline every hour.

Inhibition Studies

These experiments were carried out in a similar fashion as the trans-stimulation studies except that GlySar was included as an inhibitor of intestinal PEPT1. In these studies, rats were administered, by gastric gavage, a 1 ml predose of GlyGlu (100 mM) in combination with GlySar (100 mM). After 0.5 h, a 2 ml solution containing gabapentin (10 mg/kg plus radiolabeled tracer), along with GlyGlu and GlySar was dosed (100 mM each). Serial blood samples were then obtained as described previously.

Intravenous Dosing (IV)

Gabapentin was also dosed by IV administration as a means to compare oral drug performance in the absence and presence of dipeptides. For this procedure, the animals were sedated and anesthetized prior to surgical isolation of the femoral vein, to which, a 1 ml of gabapentin was administered (10 mg/kg plus radiolabeled tracer; in normal saline). Serial blood samples were then obtained as described above for oral dosing.

For all experiments (i.e., oral and intravenous), the animals were sacrificed prior to recovering from anesthesia. All studies were carried out in accordance with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the U. S. National Institutes of Health (NIH publication No. 85–23, revised in 1985).

Analytical Methods

Blood samples were prepared according to the Packard Solvable method (15), from which a 100-μl aliquot of sample was mixed 15 ml of scintillation cocktail (Ultima Gold, Perkin Elmer, Boston, MA) and then counted on a Beckman LS 6000 liquid scintillation counter.

Pharmacokinetic and Statistical Analyses

Pharmacokinetic variables were determined by a non-compartmental analysis using Pharsight WinNonLin 5.0 (Mountain View, CA) and included C_{max} (maximal blood concentration), t_{max} (time to maximal blood concentration), $AUC_{0-6\ h}$ (area under the blood concentration vs. time curve from 0–6 h), and $t_{1/2,\lambda z}$ (terminal half-life of the drug). The

AUC was determined by the linear up-log down method and does not include extrapolation to infinity. Data are reported as mean ± SE (n = 4), unless stated otherwise. GraphPad Prism 4.0 (San Diego, CA) was used to perform one-way ANOVA with Dunnett's post-test for pairwise comparisons, relative to control. A probability of $p \leq 0.05$ was considered statistically significant.

RESULTS

Effects of GlyGlu on Gabapentin Absorption and Systemic Exposure

To assess the effect of GlyGlu on gabapentin pharmacokinetics, the drug was dosed alone by IV administration (GB_{iv}), alone by gastric gavage ($GB_{control}$), or in combination with the dipeptide by gastric gavage ($GB_{+GlyGlu}$). As shown in Fig. 2, blood concentrations after oral administration of gabapentin (alone or in combination with 100 mM GlyGlu) were substantially lower than blood concentrations produced after IV dosing of drug, especially during the first 2 h, demonstrating incomplete systemic availability. This figure also shows that the blood concentration-time profile of orally administered gabapentin was significantly higher when dosed in combination with GlyGlu (i.e., from 15 min to 2 h; $p < 0.05$), as compared to when gabapentin was given alone. As a result, the $AUC_{0-6\ h}$ of orally administered gabapentin, when referenced to the $AUC_{0-6\ h}$ of IV gabapentin, increased from 47 to 79% in the presence of GlyGlu (Fig. 3). Likewise, GlyGlu coadministration produced an 86% increase in the C_{max} of gabapentin, as compared to control (Fig. 4). Although there was a tendency for a reduced t_{max} of gabapentin in the presence of GlyGlu, the change was not statistically significant (Fig. 5).

The effect of GlyGlu on gabapentin blood levels is believed to be through the trans-stimulation of $b^{0,+}$ exchange as delineated in the *in situ* RIP studies (7) and is consistent with the mechanism proposed by Wenzel *et. al.* (15). In theory, any dipeptides containing neutral and cationic

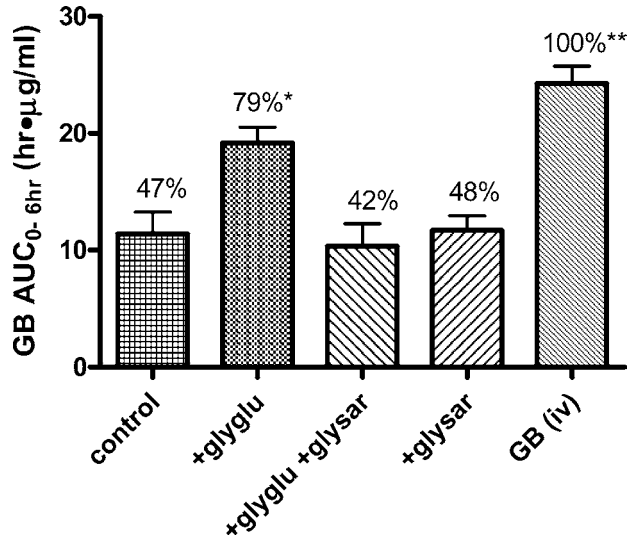

Fig. 3. Area under the blood concentration vs. time curve (AUC_{0-6hr} in hr•µg/ml) for gabapentin (GB) in rats following oral administration of GB alone (control, 10 mg/kg) or in combination with GlyGlu and/or GlySar (100 mM each). Data are presented as mean ± SE ($n = 4$), where control = 11.40 ± 1.88; +glyglu* = 19.18 ± 1.36; +glyglu + glysar = 10.30 ± 1.96; +glysar = 11.70 ± 1.24; IV** = 24.28 ± 1.49. *$p < 0.05$ and **$p < 0.01$, as compared to control.

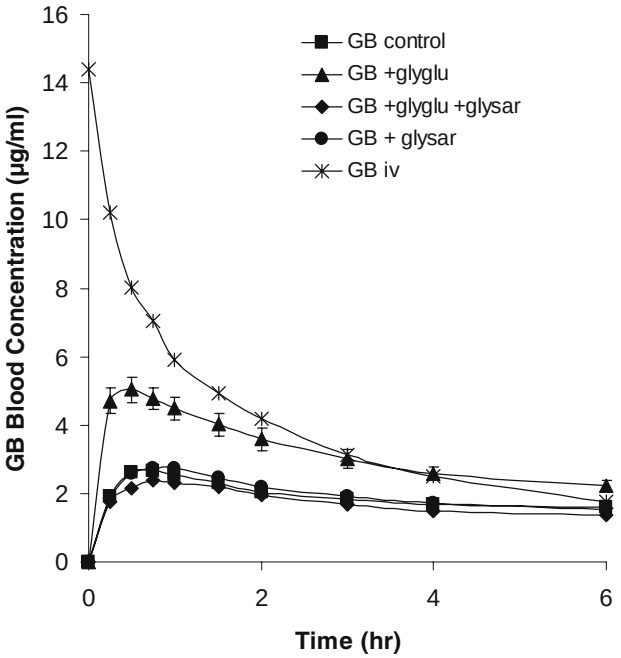

Fig. 2. Blood concentration vs. time profile of gabapentin (GB) in rats following oral dosing of GB alone ($GB_{control}$, 10 mg/kg) and in combination with GlyGlu and/or GlySar (100 mM each), and alone after IV dosing (GB_{iv}, 10 mg/kg). Data are presented as mean ± SE ($n = 4$). $GB_{control}$ values were significant lower than $GB_{+glyglu}$ (0.25–2 h; *$p < 0.05$) and GB_{iv} (0–2 h; **$p < 0.01$).

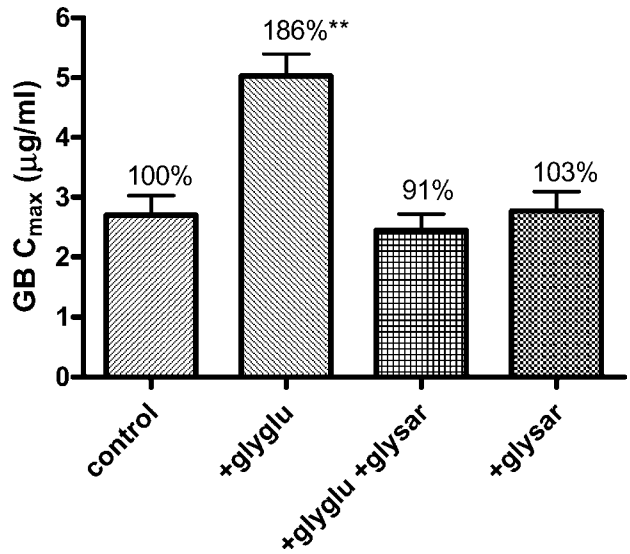

Fig. 4. Maximum blood concentration (C_{max} in µg/ml) of gabapentin (GB) in rats following oral administration of GB alone (control, 10 mg/kg) or in combination with GlyGlu and/or GlySar (100 mM each). Data are presented as mean ± SE ($n = 4$), where control = 2.70 ± 0.33; +glyglu** = 5.03 ± 0.37; +glyglu + glysar = 2.45 ± 0.27; +glysar = 2.78 ± 0.32. **$p < 0.01$, as compared to control.

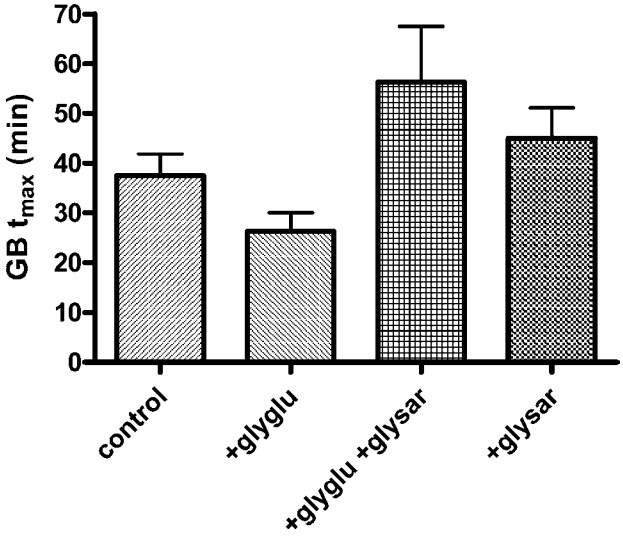

Fig. 5. Time of peak blood concentration (t_{max} in min) of gabapentin (GB) in rats following oral administration of GB alone (control, 10 mg/kg) or in combination with GlyGlu and/or GlySar (100 mM each). Data are presented as mean ± SE ($n = 4$), where control = 38 ± 4; +glyglu = 26 ± 4; +glyglu + glysar = 56 ± 12; +glysar = 45 ± 6. There were no statistical significances vs. that of control ($p > 0.05$).

substrates such as arginine, lysine, alanine, leucine, and glutamine should be able to stimulate $b^{0,+}$ uptake. However, while 80–90% of dipeptidases are found intracellularly, approximately 10–15% are located at the brush-border membrane (16). This raises the concern that extracellular dipeptide digestion could create a competitive environment for gabapentin absorption at the brush-border membrane. This was observed previously in the *in situ* RIP model whereby 20 mM GlyArg had a diminishing effect on gabapentin permeability versus the control (data not shown). Therefore, stability is a very important issue that requires consideration. However, in our studies, we do not believe that GlyGlu encountered significant metabolism at the brush-border membrane otherwise we would have observed a reduction instead of an elevation in gabapentin absorption. Our results argue against GlyGlu instability being a major factor. Moreover, GlyGlu was chosen because neither glycine nor glutamate are substrates of $b^{0,+}$ and therefore, any metabolic product at the brush-border membrane would not compete with gabapentin for uptake. The mechanism by which GlyGlu enhances gabapentin intestinal permeability involved the uptake by the PEPT1 transporter. Intracellularly, the dipeptide is hydrolyzed to Gly and Glu. The latter anionic substrate (Glu) is converted to a neutral amino acid (Gln) by glutamine synthase which in turn provided the driving force for enhanced gabapentin exchange across $b^{0,+}$.

In addition to the stability issue, the nonspecific effect of GlyGlu on gabapentin permeability was also considered because of the high dipeptide loading (100 mM). However, this value is well within the physiological range of daily protein consumption for both humans and rats. According to Ganapathy *et. al.* (17) and Daniel (18), the normal physiological concentration of dietary protein following intake in humans ranges from 120–145 mM. In rats, the daily consumption of protein and water is on the average of 3 g and 30 ml, respectively (19). Thus, the equivalent of 3 g of GlyGlu (m.w. of 204) in 30 ml of water would equate to about 490 mM, an *in vivo* concentration that is substantially higher than the 100 mM used in the present experiments. Additionally, in the *in situ* perfusion model, we have assessed the permeability of mannitol and gabapentin in the presence of a high solute load of amino acids and dipeptide (20 mM each of Ala, Leu, Gln, Glu and GlyGlu) and saw a significant stimulation of gabapentin P_{eff} ($p < 0.01$) but no effect on mannitol permeability. The *in situ* RIP model is highly reflective of an *in vivo* environment encountered following oral drug administration, i.e., preserved microclimate, intact intestinal blood supply, enzymes and brush border transporters (20–22). Therefore, we did not feel it was necessary to repeat the mannitol assessment for nonspecific influences in the *in vivo* model when none was observed in the *in situ* model. Lastly, a nonspecific effect is very unlikely since GlySar (at 100 mM) caused no change in gabapentin absorption yet the GlyGlu effect was completely reversed by GlySar (see below).

Effects of GlySar on Gabapentin Absorption and Systemic Exposure

GlySar had a significant effect when added to the orally administered admixture of gabapentin plus GlyGlu. In this regard, values for $AUC_{0-6\ h}$ and C_{max} (i.e., for $GB_{+GlyGlu+GlySar}$) reverted back to that of control (see Figs. 3 and 4). This change was not due to GlySar, per se, since the dipeptide by itself had no significant influence on gabapentin pharmacokinetics (i.e., $GB_{control}$ vs. $GB_{+GlySar}$). As shown in Fig. 2, the systemic exposure of gabapentin was significantly enhanced when gabapentin is coadministered with GlyGlu. However, the blood concentration-time profiles of gabapentin were not distinguishable when drug was administered alone or in the presence of GlySar (with or without GlyGlu being present). Thus, GlySar effectively hindered the positive influence of GlyGlu on gabapentin absorption, suggesting a competition between the two dipeptides for PEPT1-mediated uptake into the intestinal cell. The presence of GlySar appears to have completely abolished the effect of GlyGlu. However, the result is not likely a consequence of complete inhibition of GlyGlu loading through PEPT1. It is speculated that perhaps a threshold exists in which the generated intracellular glutamine must reach in order to significantly affect gabapentin permeability. Therefore, the presence of GlySar may have impeded GlyGlu loading and thus hindered the glutamine levels from reaching the required level to influence gabapentin uptake. This was observed in the *in situ* RIP model when 5–10 mM GlyGlu loading was not successful at stimulating gabapentin uptake versus 20 mM (data not shown).

The calculated kinetic parameters of gabapentin, alone and in combination with various dipeptide combinations, are captured in the legends to Figs. 3, 4, and 5. AUC_{0-inf} values (from zero to infinity) were not determined because of the unacceptably large contribution of extrapolated areas. Interestingly, the terminal half-life of gabapentin following an intravenous dose (3.4 h) was much smaller than values calculated after oral dosing (about 7.6 h, in the absence or

presence of dipeptides). The reason for this difference is unclear, but may reflect a rate-limited oral absorption of drug.

DISCUSSION

Our previous investigations in the *in situ* RIP model established, for the first time, a mechanism by which dipeptide (i.e., GlyGlu) loading through PEPT1 could improve the effective permeability of an amino acid-like drug (i.e., gabapentin) by trans-stimulating $b^{0,+}$ exchange (7). While the RIP model is widely used as a preclinical tool to predict human drug absorption (22), it is not without limitations and extrapolation to equivalent *in vivo* results must be substantiated. As a result, we have attempted to corroborate our initial findings using an *in vivo* rat model. Under physiologic, *in vivo* conditions, we were able to conclusively demonstrate that the luminal presence of GlyGlu could positively enhance gabapentin absorption and, consequently, translate into a significant improvement in the drug's $AUC_{0-6\ h}$ and C_{max}. Since the t_{max} and terminal half-life of gabapentin were unaffected by dipeptide loading, it is very unlikely that disposition was a contributing factor to these changes. Further, a dispositional change, e.g., through inhibition of renal PEPT1 (or PEPT2) by GlyGlu, would result in a reduced AUC and Cmax, which is not observed. The influence of GlyGlu on gabapentin pharmacokinetics is believed to occur through an absorptive mechanism. Our *in vivo* rat data are consistent with the RIP results which indicate that the enhanced absorption of gabapentin is a result of an increased intracellular pool of neutral amino acids that, in turn, trans-stimulates the uptake of extracellular drugs through system $b^{0,+}$. Moreover, the *in vivo* rat model also confirmed the positive influence of GlyGlu on gabapentin absorption and the negative impact of GlySar on this absorption process. It is believed that GlySar competes with GlyGlu for PEPT1-mediated uptake and, consequently, minimizes the intracellular amino acid substrates (produced after dipeptide hydrolysis) required for $b^{0,+}$ exchange.

The presence of food may cause oral drug absorption to be reduced, delayed, increased, or accelerated (23). It is important to consider food effects because of the potential impact on bioavailability, drug exposure, response and toxicity. Predicting an effect of food on drug intestinal uptake remains difficult and unreliable to this day, necessitating the evaluation of each drug for the presence of a food interaction. This study was not designed to specifically test if food by-products might interfere with the absorption of gabapentin in a clinical setting but to elucidate, *in vivo,* a mechanism-based interaction by which intestinal drug absorption can be influenced by a component of the meal. Indeed, Gidal *et al* (24) reported that the C_{max} of gabapentin was significantly enhanced (by 38%) when the drug was administered after a high-protein meal. At the time, they speculated that the large amino acid load delivered with the meal enhanced gabapentin absorption via trans-stimulation of the amino acid transport system in intestine.

In conclusion, the current findings validate our previous results from *in situ* RIP studies and establish, for the first time, a functional interaction between the activities of PEPT1 and amino acid exchange in the enhanced absorption of gabapentin *in vivo.* Moreover, these studies provide insight into a new mechanism by which a dipeptide (possibly from a protein-rich meal) might influence the biopharmaceutical properties of an amino acid-like drug.

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