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ORIGINAL ARTICLE

# Effect of Excessive Serotonin on Pharmacokinetics of Cephalexin after Oral Administration: Studies with Serotonin-Excessive Model Rats

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## Abstract

**Purpose** Serotonin (5-HT) is important for gastrointestinal functions, but its role in drug absorption remains to be clarified. Therefore, the pharmacokinetics and oral absorption of cephalexin (CEX) were examined under 5-HT-excessive condition to understand the role of 5-HT.

**Methods** 5-HT-excessive rats were prepared by multiple intraperitoneal dosing of 5-HT and clorgyline, an inhibitor for 5-HT metabolism, and utilized to examine the pharmacokinetics, absorption behavior and the intestinal permeability for CEX.

**Results** Higher levels of 5-HT in brain, plasma and small intestines were recognized in 5-HT-excessive rats, where the oral bioavailability of CEX was significantly enhanced. The intestinal mucosal transport via passive diffusion of CEX was significantly increased, while its transport via PEPT1 was markedly decreased specifically in the jejunal segment, which was supported by the decrease in PEPT1 expression on brush border membrane (BBM) of intestinal epithelial cells. Since no change in antipyrine permeability and significant increase in FITC dextran-4 permeability were observed in 5-HT-excessive rats, the enhanced permeability for CEX would be attributed to the opening of tight junction, which was supported by the significant decrease in transmucosal electrical resistance. In 5-HT-excessive rats, furthermore, total body clearance of CEX tended to be larger and the decrease in PEPT2 expression on BBM in kidneys was suggested to be one of the reasons for it.

**Conclusions** 5-HT-excessive condition enhanced the oral bioavailability of CEX in rats, which would be attributed to the enhanced permeability across the intestinal mucosa via passive diffusion through the paracellular route even though the transport via PEPT1 was decreased.

**Keywords** Serotonin · PEPT1 · PEPT2 · Cephalexin · Oral absorption

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## Introduction

Movements and functions of the small intestine are well known to be regulated by intrinsic neurons of the enteric nervous system (ENS) and by extrinsic sympathetic, parasympathetic and sensory neurons derived from the central nervous system (CNS) [1]. ENS is recognized as an independent integrative system with structural and functional properties similar to those of CNS and functions independently without extrinsic neuronal influences [2]. It has been intensively examined how ENS is involved in the regulation of the smooth muscle [3–8] and the transport of water and/or electrolytes [7, 9–14]. Specifically, serotonin (5-hydroxytriptamine, 5-HT), of which over 95% found in the body is mainly contained in the enterochromaffin cells of intestinal epithelium and also in neurons of myenteric plexus [15], has recently drawn much attention on its importance in the gastrointestinal functions as a paracrine messenger and neurotransmitter [16–18]. However, the role of 5-HT in gastrointestinal physiology, pathophysiology and/or ENS remains to be fully understood because 5-HT exerts the diverse effects on a wide range of physiological functions through an imposing number of 5-HT receptors [19]. The interaction between ENS and intestinal epithelium including the role of 5-HT, which is important for the intestinal function, was not fully understood yet [20].

Furthermore, it has recently been considered that several serious bowel diseases such as inflammatory bowel disease (IBD) and irritable bowel syndrome (IBS) have been associated with ENS disorders [16, 21–23] and the clarification of the relationship between these diseases and ENS dysfunction has also been expected from both pathological and pharmacological aspects [23]. Specifically, it has recently been suggested that 5-HT would be related with several serious bowel diseases such as IBS and celiac diseases [24]. 5-HT levels in the inflamed mucosa were markedly decreased in human IBD such as ulcerative colitis (UC) and Crohn's disease (CD) [25]. To the contrary, it has been suggested that the 5-HT is elevated in IBS and celiac disease [15, 26]. Furthermore, IBD is associated with the functional depletion of ENS [23] and the abnormalities of ENS including 5-HT levels should be deeply involved in these gastrointestinal diseases [22, 23, 25], although the causal association has not been fully clarified yet.

On the other hand, it remains to be clarified how drug absorption from small intestine is regulated by ENS and/or how drug absorption is affected under gastrointestinal diseases related with ENS disorders, since studies for the neural effect on drug absorption from small intestine are still very limited [7, 13, 27]. Thus, it is very important to find out the changes in intestinal functions, specifically in the absorptive function for drugs, under varied physiological conditions related with ENS disorder for the efficient oral medication.

Although it is very difficult technically to clarify the interaction between ENS and intestinal mucosa [20], we have already performed several studies to figure out the neural regulation of drug absorption from the small intestine, [28–31]. The effects of adrenergic stimulation on the intestinal absorption and transport of a small molecular organic anion, phenol red [28] and cation, rhodamine-123 [31] were examined by utilizing a vascular-luminal perfused preparation and an isolated jejunal sheet. Although its effect on small molecule transport via passive diffusion is still controversial [28, 30, 31], the adrenergic stimulation suppressed the transport of a large molecular compound across Caco-2 cells monolayer [30]. The luminal secretion of rhodamine-123 was also suppressed via the decrease in the expression level of P-glycoprotein (P-gp), a well-known ATP-dependent transporter extruding so many clinically important drugs [32], on brush border membrane by the adrenergic stimulation [31]. As for 5-HT, considering its levels in UC and CD [25], chronic depletion of 5-HT significantly enhanced P-gp activity of the rat small intestine due to the enhanced expression of P-gp on brush border membrane and the passive transport via paracellular pathway [29].

In the current study, to obtain some information on drug absorption under the condition where 5-HT is elevated such as IBS and celiac disease [15, 26], we tried to prepare 5-HT-excessive model rats. Although we referred to several reports on the preparation of 5-HT-excessive rats [33, 34], all the rats intraperitoneally injected with 5-hydroxy-L-tryptophan (5-HTP, 100 mg/kg) and clorgyline (2 mg/kg) [33] died from 75 to 90 min after the injection, revealing that the 5-HT-excessive condition should be too severe to exert any in vivo studies to investigate the effect of excessive 5-HT on drug absorption. Furthermore, 5-HTP is a precursor of 5-HT and the generation of 5-HT should be changeable dependent on the activities of tryptophan hydroxylase 1 and 2 [35]. Since we would like to exclude a possible effect of fluctuation of biosynthesis of 5-HT, we selected the way of the direct injection of 5-HT and the inhibition of its metabolism to make a 5-HT excessive condition in the current study. After several preliminary experiments, we prepared 5-HT-excessive model rats, which allows us to exert both in vivo and in vitro studies to examine drug absorption from the small intestine. Then, we tried to estimate the effect of excessive 5-HT on the pharmacokinetics of cephalexin, which is absorbed via PEPT1 and passive diffusion from the small intestine [36], after oral administration.

## Materials and Methods

### Materials

Cephalexin anhydrate (CEX), glycyl-sarcosine (Gly-Sar), serotonin hydrochloride (5-HT), clorgyline (N-methyl-N-propargyl-3-(2,4-dichlorophenoxy) propylamine hydro-chloride), antipyrine, 4-dimethylamino-antypyrine, 5-methoxy-DL-tryptophan and FITC-dextran 4 (FD-4) were purchased from Sigma Chemical Co. (St. Louis, MO). All other chemicals and reagents were analytical grade commercial products.

### Animals

Male Wistar rats (7–9 weeks, Charles River Laboratories Japan, Yokohama, Japan), maintained at 25°C and 55% humidity under 12-h lighting condition (8:00–20:00), were allowed free access to standard laboratory chow (Clea Japan, Tokyo, Japan) and water. Rats were randomly assigned to each experimental group. Every animal experiment was started around 14:00 to avoid the effect of diurnal change in PEPT1 activity [37–39]. Our investigations were performed after approval by our local ethical committee at Okayama University and in accordance with "Principal of Laboratory Animal Care (NIH publication #85-23)".

### Preparation of 5-HT-Excessive Model Rats

5-HT-excessive model rats were prepared by intraperitoneally injecting 5-HT (5 mg/kg) and clorgyline, a monoamine oxidase-A (MAO-A) inhibitor, (2 mg/kg) dissolved in saline for 4 days. All the experiments were performed around 24 h after the last injection of 5-HT and clorgyline unless otherwise specified. For control rats, saline was intraperitoneally injected instead of 5-HT and clorgyline solutions. 5-HT levels in the small intestine and brain were determined by the method reported by Lakshmana and Raju [40] with minor modification. At fixed period times after final intraperitoneal injection of 5-HT and clorgyline, the ileum and whole brain were removed, minced and homogenized in a 7-fold volume of 7.14% ascorbic acid containing isoproterenol as an internal standard. The resulting mixture was, moreover, homogenized with the equivalent volume of 30% $HClO_4$. All the processes described above were performed on ice. After centrifugation at 9000×*g* for 15 min, the supernatant was filtered through a millipore filter (0.22 µm, Millex-HV; Millipore Corporation, Billerica, MA). As for plasma concentrations, blood sampled at fixed period times were centrifuged at 8000×g for 10 min to obtain plasma samples, which were deprotenized by methanol containing 100 µM 5-methoxy-DL-tryptophan as an internal standard. Then, aliquots of the resulting supernatant were injected onto the HPLC system.

### In Vivo Oral and Intravenous Administration Studies

One day before drug administration, the jugular vein of a rat was cannulated with vinyl tubing (ca 20 cm) (SV-45, i.d. 0.58 mm, o.d. 1.0 mm, Natsume, Tokyo) connected with SR tube (1.5 cm) (i.d. 0.5 mm, o.d. 1.0 mm, Shin-Etsu Polymer Co., Ltd., Tokyo) under anesthesia. In the case of oral administration, CEX dissolved in saline was intragastrically administered at a dose of 5 mg/5 ml/kg, corresponding to a standard therapeutic dose. For intravenous administration, CEX dissolved in saline was administered from the tail vein at the dose of 5 mg/ml/kg. Blood samples were periodically taken from the cannulated jugular vein. Plasma obtained by centrifugation was deproteinized by methanol and the resulting supernatant was used for HPLC analysis.

### In Vitro Intestinal Transport Study

The jejunal segment 30 cm below the ligament of Treitz and the ileal segment 30 cm above ileocecal junction were removed under anesthesia and rinsed in ice-cold saline. After the segments were opened along the mesenteric border, intestinal contents were washed out with ice-cold saline. Immediately, the muscularis propria was stripped off, and three to four jejunal or ileal sheets approximately 5 cm in length without Peyer's patches were prepared [29]. The intestinal sheet prepared above was mounted in a diffusion chamber (Corning Coaster Japan, Tokyo) with a 1.25-$cm^2$ exposed area. Ringer's solution, containing 1.2 mM $NaH_2PO_4$, 125 mM NaCl, 5 mM KCl, 1.4 mM $CaCl_2$, 10 mM $NaHCO_3$, and 2 mg/ml D-glucose was gassed with 95% $O_2$ and 5% $CO_2$ for 15 min and adjusted to pH 6.5, 7.0 or 7.4 with a few drops of 1 N HCl or NaOH. Then Ringer's solution (pH 6.5 for jejunum; pH 7.0 for ileum) was placed into the apical compartment and pH 7.4 Ringer's solution was placed into the basal compartment [36]. During the entire experiment, Ringer's solution in both sides was gassed with 95% $O_2$ and 5% $CO_2$ and maintained at 37°C. It was confirmed that the pH value of Ringer's solution was kept around 6.5, 7.0 or 7.4 throughout the transport studies. The transmucosal electrical resistance (TER) was calculated following Ohm's law. After preincubation for 25 min to stabilize the electrical condition of tissues, the solution in the apical side was exchanged with the drug solution (500 µg/mL CEX, 0.5 µmol/mL antipyrine or 0.2 µmol/mL FD-4). Once the transport experiments started, the solutions in both sides were circulated by gas lift with 95% $O_2$ and 5% $CO_2$ throughout the transport studies. Aliquots of the solution in the basal side were sampled at 10-min intervals for 90 min. An equal volume of Ringer's solution was immediately added to the basal side after each sampling. In the case of inhibition studies for CEX, Gly-Sar (50 µmol/mL), a typical substrate for PEPT1 [37], was added with CEX (500 µg/mL≒1.37 µmol/mL) to the apical side. Drug concentrations in the basal side were determined by HPLC.

### Western Blot Analysis

Western blot analysis was performed utilizing brush border membrane fraction of rat small intestinal mucosa or kidney. Brush border membrane vesicles (BBMVs) were prepared by the method reported by Kessler et al. [41] with minor modification [29] or the method reported by Wilfong and Neville, Jr. [42] for the small intestine or kidney, respectively. Final BBMVs were enriched in the activity of alkaline phosphatase compared with the corresponding tissue homogenate (Jejunum: control, 13.8 ± 3.2; 5-HT-excessive, ca 13.3 ± 3.0. Ileum: control, 7.3 ± 2.1; 5-HT-excessive, 6.8 ± 2.8. Kidney: control, 8.0 ± 0.4; 5-HT-excessive, 8.3 ± 0.7). All the equipment and chemicals used in the Western blot analysis were obtained from Bio-Rad (Hercules, CA) unless otherwise specified. BBMVs resuspended in the sample buffer were separated by SDS-PAGE using 7.5% Mini-Protean TGX gel (Bio-Rad) according to the method of Laemmli [43], and transferred to nitrocellulose membranes. The blots were blocked with phosphate-buffered saline containing 5% nonfat milk by 1.5-h incubation at room temperature, and incubated with the PEPT1 polyclonal antibody, H-235 (Santa Cruz Biotechnology, Inc., Santa Cruz, CA), PEPT2 polyclonal antibody (Bioss, Inc., Woburn, MA), or villin polyclonal antibody, C-19 (Santa Cruz Biotechnology, Inc.). The blots were then incubated with anti-rabbit HRP antibody (Thermo Fisher Scientific, Waltham, MA) or anti-goat HRP antibody (Santa Cruz Biotechnology, Inc.). The blots were developed with an CL kit (GE healthcare life sciences Japan, Tokyo) and bands were detected by ImageQuant LAS 4000 (GE healthcare life sciences). The quantification of bands was performed by densitometric analysis using the Scion image (Scion Co., Frederick, MD).

### Estimation of Gastrointestinal (GI) Transit

To estimate the possible change in GI transit under 5-HT-excessive condition, GI transit of glass beads (GB-0.07, sp.gr. 2.5 g/cm$^3$; o.d. 63–88 μm, Kenis Ltd., Osaka, Japan), an unabsorbable marker, was estimated for each segment of small intestine after oral administration, since the intestinal transit is well known not to be affected by physical state of drugs (ex. solution or powder) [44], food [45–48], size [49] nor density [49, 50]. For the stomach, however, since gastric emptying is faster for liquid than solid [44, 51, 52], phenol red solution was employed as an unabsorbable marker for estimating gastric emptying rate constant. At appropriate time periods after oral dosing of markers, whole GI tract was removed and the remaining glass beads and/or phenol red were carefully recovered by washing out with saline for stomach (s), duodenum (d), upper jejunum (uj), lower jejunum (lj), upper ileum (ui), lower ileum (li). After centrifuging the washings collected, the supernatant obtained were used for the determination of phenol red, which was performed colorimetrically at 560 nm after alkalinized. In the case of glass beads, the washings were placed into the tube where 200 μL of 3 N KOH (sp.gr. 1.168) and 800 μL of silicon oil (sp.gr. 1.05, Sigma Chemical Co.) were placed in advance. After centrifuging the tube at 47×g for 15 min, removing the oil phase and washing out KOH with distilled water, the tube was lyophilized and weighed. The remaining amount of glass beads were estimated as the difference in tube weight between before- and after-experiments [44]. Except for stomach, GI transit time was calculated as a reciprocal of GI transit clearance (CLgi) reflecting GI transit rate constant calculated by the following equation [44, 53, 54]:

$$\text{GI transit time} = \frac{1}{\text{CL}_{\text{gi}}}(\text{hr}) \tag{1}$$

$$\text{CLgi} = \frac{100}{\text{AUC}_{\text{glass}}}(\text{hr} - 1) \tag{2}$$

where AUC means the area under the recovery% of dose of glass beads – time curve and was calculated by following the trapezoidal rule.

In the case of stomach, the retention time in the stomach was calculated by a reciprocal of gastric emptying rate constant which was calculated by assuming the first-order kinetics for gastric emptying of phenol red [55].

### Analytical Method

For detection of 5-HT, antipyrine and CEX, HPLC, which consists of a model LC-20A HPLC pump (Shimadzu, Kyoto, Japan), a model of SIL-9A system controller (Shimadzu), was used at room temperature. For 5-HT, Inertsil ODS-3 column (250 × 4.6 mm i.d., GL Sciences, Inc. Tokyo, Japan) was used and the mobile phase, 20 mM sodium acetate (pH 3.9), methanol, and heptane sulfonic acid, 800:200:0.1 (*v/v*) was delivered at 1.0 mL/min. 5-HT was detected by a model RF-10AxL fluorescence detector (Shimadzu) set at 349 nm (excitation) and 444 nm (emission). The standard curves from 0.1 to 10 nmol/mL gave the coefficients of variation (CV) ranged from 0.07% to 32.9%. The correlation coefficients were over 0.9968.

For antipyrine, CAPCELL PAK UG120Å (150×4.6 mm, i.d., Shiseido Co. Ltd., Tokyo) was used and the mobile phase, 20 mM phosphate buffer (pH 7.4):methanol = 65:35 (*v/v*) was delivered at 1.0 mL/min. Antipyrine was detected by a model SPD-20A UV detector (Shimadzu) set at 254 nm. Standard curves from 0.5 to 50 nmol/ml provided CV values ranged from 0.26 to 4.77%. The correlation coefficients was over 0.997.

For CEX, Inertsil ODS-3 column or Chromolith (50 × 4.6 mm, i.d., MSD K.K., Tokyo) was used for in-vivo sample or in-vitro sample, respectively. The mobile phase for plasma sample was 10 mM acetate buffer (pH 6.0):methanol (70:30; *v/v* for in-vivo sample, 85:15; *v/v* for in-vitro sample) delivered at 1.0 mL/min. CEX was detected by a model SPD-20A UV detector set at 260 nm. Standard curves from 0.5 to 20 mg/mL gave CV values ranged from 5.35 to 8.48%. The correlation coefficients were over 0.9982.

FD-4 were determined fluorospectrophotometrically at 485 nm for excitation and at 515 nm for emission (F4500 fluorescence spectrophotometer, Hitachi, Tokyo).

### Pharmacokinetic Analysis

For in vivo intravenous administration study, pharmacokinetic parameters describing the plasma concentration–time profile of CEX after intravenous administration were obtained based on a two-compartment model by the non-linear least-squares regression program MULTI [56]. The following equation was utilized to express the plasma concentration-time profile of CEX:

$$C_p = A \cdot e^{-\alpha \cdot t} + B \cdot e^{-\beta \cdot t} \tag{3}$$

where α and β are rate constants for the distribution phase and elimination phase, respectively. A and B are hybrid constants shown as $D \cdot (\alpha - k_{21})/Vc/(\alpha - \beta)$ and $D \cdot (k_{21} - \beta)/Vc/(\alpha - \beta)$, respectively. D, $k_{21}$ and Vc mean the dose, first-order rate constant from peripheral to central compartment and distribution volume in central compartment, respectively. AUC and $CL_{total}$ were calculated by the following equations:

$$AUC = \frac{A}{\alpha} + \frac{B}{\beta} \tag{4}$$

$$CL_{total} = \frac{D}{AUC} \tag{5}$$

For in vivo oral administration study, the maximum concentration, $C_{max}$, was the highest concentration observed and the time to reach $C_{max}$ was defined as $T_{max}$. Area under the plasma concentration – time curve, AUC, from 0 to 6 h was calculated following the trapezoidal rule. Mean residence time after oral administration, $MRT_{po}$, and mean absorption time, MAT, were calculated based on the statistical moment theory. F values were calculated by utilizing AUC values obtained after intravenous administration.

For in vitro intestinal transport study, the cumulative amounts of drugs transported to the basal side was calculated by the following equation:

$$Q(t_k) = \sum_{k=1}^{n} C(t_{k-1}) + V \cdot C(t_k) \tag{6}$$

where $Q(t_k)$ and $C(t_k)$ mean the cumulative amount transported to the basal side and drug concentration in the basal side at time $t_k$, respectively. V reveals the volume of solution in the basal side. Then, the apparent permeability coefficient, $P_{app}$, was calculated by the following equation:

$$P_{app} = \frac{dQ}{dt} \cdot \frac{1}{A \cdot C_0} \tag{7}$$

where dQ/dt, A and $C_0$ mean the permeation rate, exposing surface area and initial drug concentration in the apical side, respectively.

### Statistical Analysis

Results are expressed as the mean with S.E. Analysis of variance (ANOVA) was used to test the statistical significance of difference among groups. Statistical significance in the difference of the means was determined with Tukey's test or Student's *t* test for multiple or single comparison of experimental groups, respectively.

## Results and Discussion

### 5-HT Level in 5-HT-Excessive Rat

In the rats where 5-HT and clorgyline were intraperitoneally injected single or four times, 5-HT levels in the small intestine, brain and plasma were determined (Fig. 1). In the small intestine, very high levels of 5-HT were observed just after injection, but the higher levels were kept for a few hours after 4th injection (Fig. 1(A)). Since serotonin transporter (SERT) is expressed on both apical and basal membranes of intestinal epithelial cells [57], we had thought that 5-HT taken up into the epithelial cells would be accumulated by the inhibition of its metabolism by clorgyline. However, it was found that the effect did not last for a long time. On the other hand, the higher levels of 5-HT were kept for longer time in the brain (Fig. 1(B)) and plasma (Fig. 1(C)), although the increase in 5-HT level in brain was unexpected because blood brain barrier usually prevents 5-HT from penetrating the brain from the systemic circulation and SERT effluxes 5-HT from the brain [58]. Since SERT is known to be expressed in the luminal side of endothelial cells of brain microvasculature [59], the SERT might have functioned for the uptake of 5-HT; and/or clorgyline might have been effectively taken up into the brain where clorgyline might also have inhibited the metabolism of 5-HT effectively, leading to the saturation of efflux via SERT and the accumulation of 5-HT. Results shown in Fig. 1 indicate that the long-lasting higher levels of 5-HT were not acquired in the small intestine, but the relatively long-lasting higher levels were done in plasma, indicating that organs and tissues including the small intestine were continuously exposed by higher levels of 5-HT from the blood circulation. The administration of 5-HT and clorgyline caused the high levels of 5-HT in the brain, plasma and small intestine, but the pattern of enhancement was dependent on each organ and plasma. The difference among the two organs and plasma is very interesting and worth studying the mechanisms behind this phenomenon. However, since the current study is focusing on the effect of excessive 5-HT on the pharmacokinetics and oral absorption behavior of CEX, we will, next, try to evaluate them utilizing the 5-HT-excessive rats.

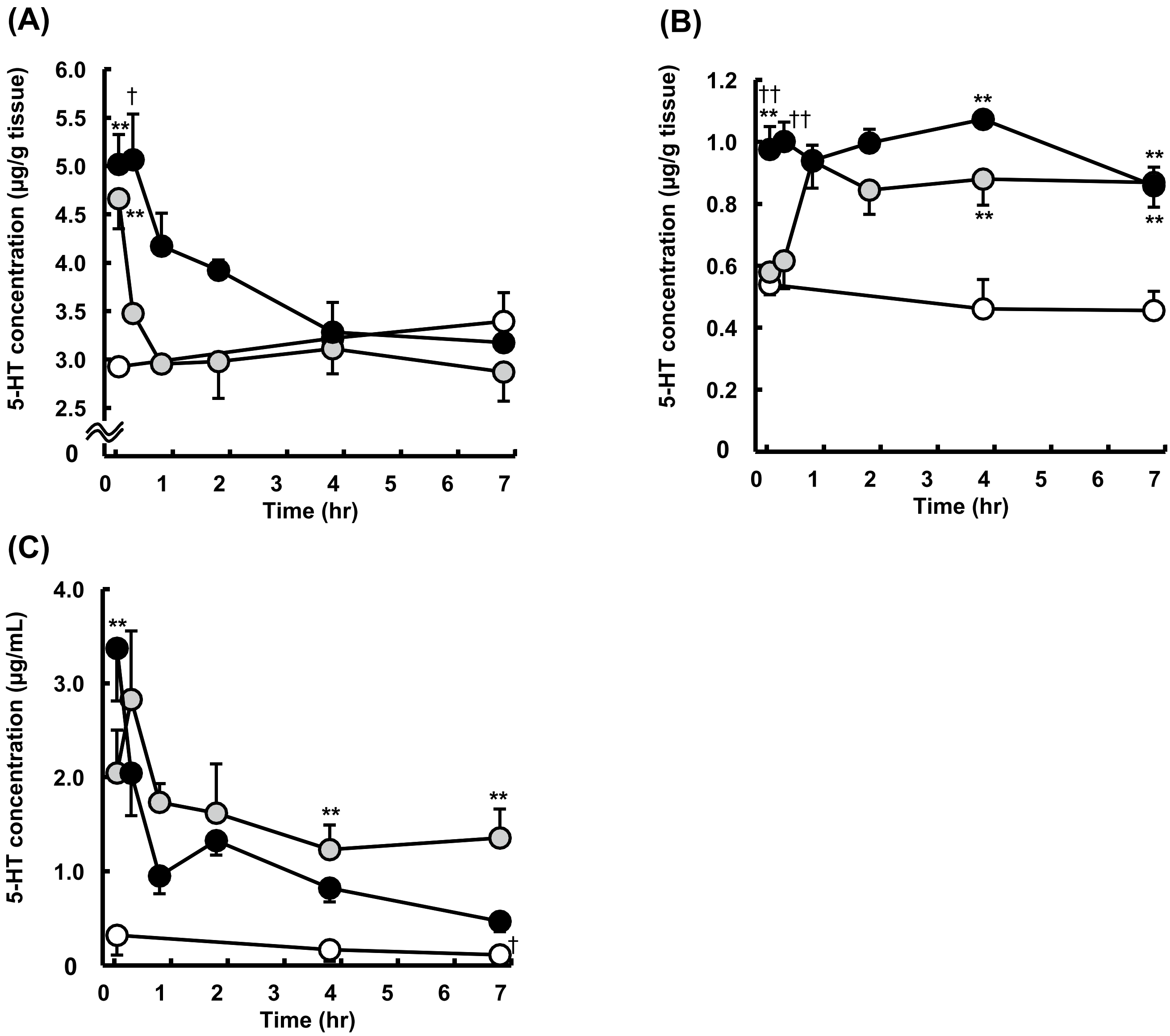


**Fig. 1** Small intestine (**A**), brain (**B**) and plasma (**C**) levels of 5-HT in 5-HT- excessive model rats. Results are expressed as the mean with S.E. bar of 3 to 8 experiments. **, $p < 0.01$ compared with control. ††, $p < 0.01$; †, $p < 0.05$ compared with single dose of 5-HT and clorgyline. Keys: ○, control; ◯ (gray), single dose; ●, four doses of 5-HT and clorgyline.

## In Vivo Pharmacokinetics of CEX in 5-HT-Excessive Rats

Plasma concentration – time profiles of CEX after intravenous administration indicate that the elimination of CEX at β phase was significantly faster in 5-HT excessive rat (Fig. 2(A)). Table 1 also reveals that β and $MRT_{iv}$ were significantly larger and smaller, respectively, and $CL_{total}$ and $k_{el}$ tended to be larger in 5-HT-excessive rats than control rats. On the other hand, no difference in distribution volumes including $Vd_1$ was observed.

After oral administration of CEX, $C_{max}$ tended to be higher and plasma concentrations at 1.5, 2 and 4 hr. were significantly higher in 5-HT-excessive rats than those in control rats (Fig. 2(B) and Table 2). AUC was also significantly larger and the bioavailability was calculated to be as high as 96% in 5-HT-excessive rats (Table 2). These results clearly indicate that the oral absorption of CEX was significantly enhanced in 5-HT-excessive rats. Even though $MRT_{iv}$ was significantly shorter for 5-HT-excessive rats (Table 1), $MRT_{po}$ was significantly increased, resulting in the significant prolongation of MAT (Table 2). Usually, the larger value of MAT means the low value of ka, the first order absorption rate constant, because MAT is a reciprocal of ka in correspondence with the compartment model. However, in the case of CEX absorption in the 5-HT-excessive rats, the larger value of MAT would just reflect the prolongation of absorption, but do not the slowdown of CEX

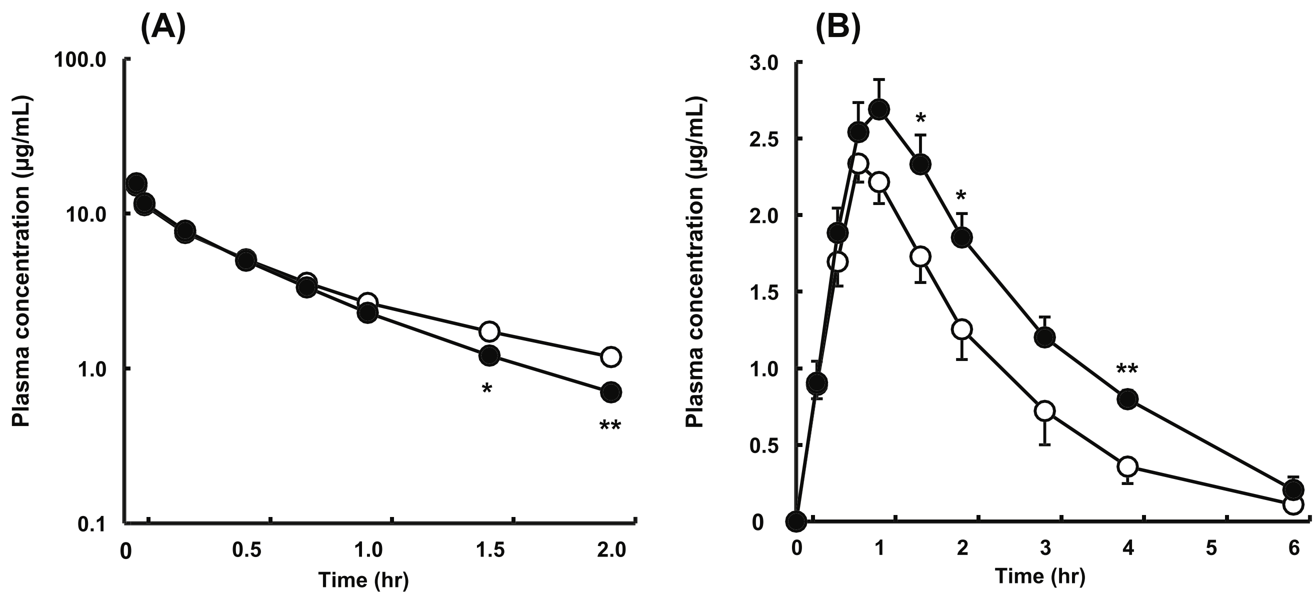


**Fig. 2** Plasma concentration – time profiles of cephalexin after intravenous (**A**) and oral (**B**) administrations into control and 5-HT-excessive rats. Results are expressed as the mean with S.E. bar of 5 to 8 experiments. **, $p<0.01$; *, $p<0.05$ compared with control. Keys: ○, control rat; ●, 5-HT-excessive rat.

absorption. According to finite absorption time (FAT) concept [60], which has recently been proposed, $T_{max}$ would be coincided with the time when the absorption is terminated, although $T_{max}$ would usually be the time when absorption (input) rate has been equal to the elimination rate. FAT concept also suggests that the larger values of $k_{el}$ or β would indicate smaller values of FAT [60]. Since $T_{max}$ of CEX for 5-HT excessive rats (1.00 hr., Table 2) tended to be delayed compared with control rats (0.83 hr., Table 2), the termination of absorption would be delayed based on FAT concept, which would have led to the increase in CEX absorption for 5-HT excessive rats. Furthermore, the large value of β for CEX (0.85–1.17 $hr.^{-1}$, Table 1) suggests that $T_{max}$ or FAT for CEX would be less than 1.5 hr., reflecting our observed values (Fig. 2b and Table 2). Considering that the substantial absorption of CEX should occur from the ileal segment and GI transit times [36], the absorption of CEX might have been terminated at some time point of the descending limb of the plasma concentration – time curve [60], but the increase in $T_{max}$ value would reflect the prolongation of absorption time.

## In Vitro Transport Study across Isolated Intestinal Sheet

To find out the reasons why oral absorption of CEX was enhanced in 5-HT-excessive rats, the in vitro transmucosal studies were performed by utilizing the jejunal and ileal sheets isolated from 5-HT excessive rats (Fig. 3). In jejunum, no change was found in $P_{app}$ of CEX (Fig. 3(A) and (B)), but the permeability of CEX across the ileal sheet was significantly enhanced under 5-HT excessive condition (Fig. 3(C) and (D)).

Since CEX is partly absorbed via PEPT1 [36], the contribution of PEPT1 to the changes in $P_{app}$ of CEX was examined by utilizing Gly-Sar as a competitive inhibitor for CEX absorption via PEPT1. In the control rats, the contribution of PEPT1 to CEX transport was very large in the jejunum (60.45 ± 4.50%), although its contribution was so small in the ileum (15.30 ± 5.86%) because of lower $H^+$ gradient, supporting our previous findings [36]. The effect of 5-HT-excessive condition would be a little bit complicated. In the jejunum, although the permeability was not changed, the contribution of PEPT1 to it was significantly decreased (26.93 ± 5.79, $p<0.01$ vs control rat) (Fig. 3(A) and (B)) , meaning the possible enhancement of CEX transport via passive diffusion. In the ileum, on the other hand, $P_{app}$ was significantly increased, but the contribution of PEPT1 was still so small under 5-HT-excessive condition (20.94 ± 5.52, N.S. vs control rat) (Fig. 3(C) and (D)). Although it apparently seems like 5-HT-excessive condition exerted the different effects for the jejunum and ileum, the transport via passive diffusion was clearly enhanced in common. As for the transport via PEPT1, the effect was not clearly observed in the ileum because the contribution of PEPT1 was so small under the physiological condition.

Then, the membrane permeability via passive diffusion was examined by utilizing antipyrine and FD-4 as a transcellular marker and paracellular marker, respectively. Figure 4 clearly indicates that 5-HT excessive condition did not affect the drug transport via transcellular route in both the jejunal and ileal segments. As for the paracellular route, the transmucosal transport of FD-4 was significantly increased in

**Table 1** Pharmacokinetic parameters of cephalexin after intravenous administration to control and 5-HT-excessive rats

| Rats | Pharmacokinetic parameters | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | A (µg/mL) | α ($hr^{-1}$) | B (µg/mL) | β ($hr^{-1}$) | AUC (µg/mL·hr) | CLtotal (mL/hr./kg) | kel ($hr^{-1}$) | $Vd_1$ (mL/kg) | $Vd_2$ (mL/kg) | $Vd_{ss}$ (mL/kg) | MRTiv (hr) |
| Control | 11.50 ± 1.48 | 7.60 ± 1.57 | 6.43 ± 0.83 | 0.85 ± 0.05 | 9.38 ± 0.53 | 542.6 ± 33.3 | 1.98 ± 0.25 | 285.4 ± 19.5 | 243.2 ± 25.1 | 528.6 ± 19.6 | 0.99 ± 0.05 |
| 5-HT excessive | 10.90 ± 1.26 | 6.65 ± 1.24 | 7.07 ± 0.87 | 1.17** ± 0.05 | 7.79 ± 0.47 | 651.7 ± 40.8 | 2.31 ± 0.18 | 287.4 ± 25.8 | 171.8 ± 2.8 | 459.2 ± 42.4 | 0.70** ± 0.03 |

Results are expressed as the mean ± S.E. of 5–6 experiments. **, $p < 0.01$, compared with Control

the both segments under 5-HT-excessive condition (Fig. 5). TER values were significantly decreased, clearly indicating that the paracellular route was expanded in both intestinal segments (Fig. 6). These results suggest that the enhancement of CEX transport via passive diffusion shown in Fig. 3 would be attributed to the increased passive transport through the paracellular route but not through the transcellular route. Although the mechanisms behind the expansion of paracellular route remain to be elucidated, the tight junction opening due to the condensation of actin-myosin II rings would be one of probable reasons [61, 62]. It is known that 5-$HT_1$, 5-$HT_2$, 5-$HT_3$, 5-$HT_4$ [63] and 5-$HT_7$ [63, 64] are localized to intestinal epithelial cells and that 5-HT increases intracellular $Ca^{2+}$ level via these receptors [65], specifically, 5-$HT_2$ [66], 5-$HT_3$ [63, 66] and 5-$HT_4$ [63, 67]. Since the elevation of intracellular $Ca^{2+}$ level would lead to the activation of myosin light-chain kinase (MLCK), the activated enzyme would have condensed the actin-myosin II rings [61, 62].

## PEPT1 Expression on Brush Border Membrane of Small Intestine

Since the contribution of PEPT1 to the intestinal transport of CEX was significantly decreased in the jejunum under 5-HT-excessive condition, the expression level of PEPT1 was examined by Western blot analysis (Fig. 7). Although no change in the expression was observed in whole mucosal homogenate for both the segments, the expression levels on brush border membrane were significantly decreased for both the segments under 5-HT-excessive condition. This observation clearly explains the reason for the significant decrease in CEX transport via PEPT1 in the jejunal segment under 5-HT-excessive condition (Fig. 3(A) and (B)). The mechanisms behind the decrease in PEPT1 expression on brush border membrane remains to be clarified, but the decrease in the trafficking of PEPT1 from an intracellular pool to the brush border membrane would be at least involved in it, considering no change in the expression level in the whole mucosal homogenate (Fig. 7). It was reported that trafficking of PEPT1 to the apical membrane was suppressed by the elevation of intracellular $Ca^{2+}$ [68] and the activation of PKC [68, 69]. As described above, 5-HT increases the intracellular $Ca^{2+}$ via several 5-HT receptors [63, 65–67]. Since the elevation of intracellular $Ca^{2+}$ leads to the activation of PKC [65, 68], 5-HT-excessive condition would have caused the decrease in PEPT1 trafficking to the brush border membrane.

It is also well known that 5-HT increases cAMP level and the elevation of cAMP is also one of the reasons for the increase in intracellular $Ca^{2+}$ [66]. $\alpha_2$-Adrenergic stimulation, which decreases cAMP [30, 31], increased the translocation of PEPT1 to the apical membrane [70]. This opposite way of PEPT1 trafficking to the apical membrane would also suggest that intracellular $Ca^{2+}$, cAMP and/or

**Table 2** Pharmacokinetic parameter of cephalexin after oral administration into control and 5-HT-excessive rats

| Rats | Pharmacokinetic parameters | | | | | |
|---|---|---|---|---|---|---|
| | Tmax (hr) | Cmax (μg/mL) | MRTpo (hr) | MAT (hr) | AUC (μg/mL·hr) | F (%) |
| Control | 0.83 ± 0.05 | 2.39 ± 0.14 | 1.77 ± 0.14 | 0.79 ± 0.14 | 5.24 ± 0.70 | 55.8 ± 7.41 |
| 5-HT excessive | 1.00 ± 0.08 | 2.73 ± 0.19 | 2.12 ± 0.08* | 1.42 ± 0.08** | 7.51 ± 0.48* | 96.4 ± 6.34** |

F, meaning the absolute bioavailability, was calculated by utilizing the results of intravenous administration study shown in Fig. 2(A) and Table 1. Results are expressed as the mean ± S.E. of 6–8 experiments. **, $p<0.01$: *, $p<0.05$, compared with Control

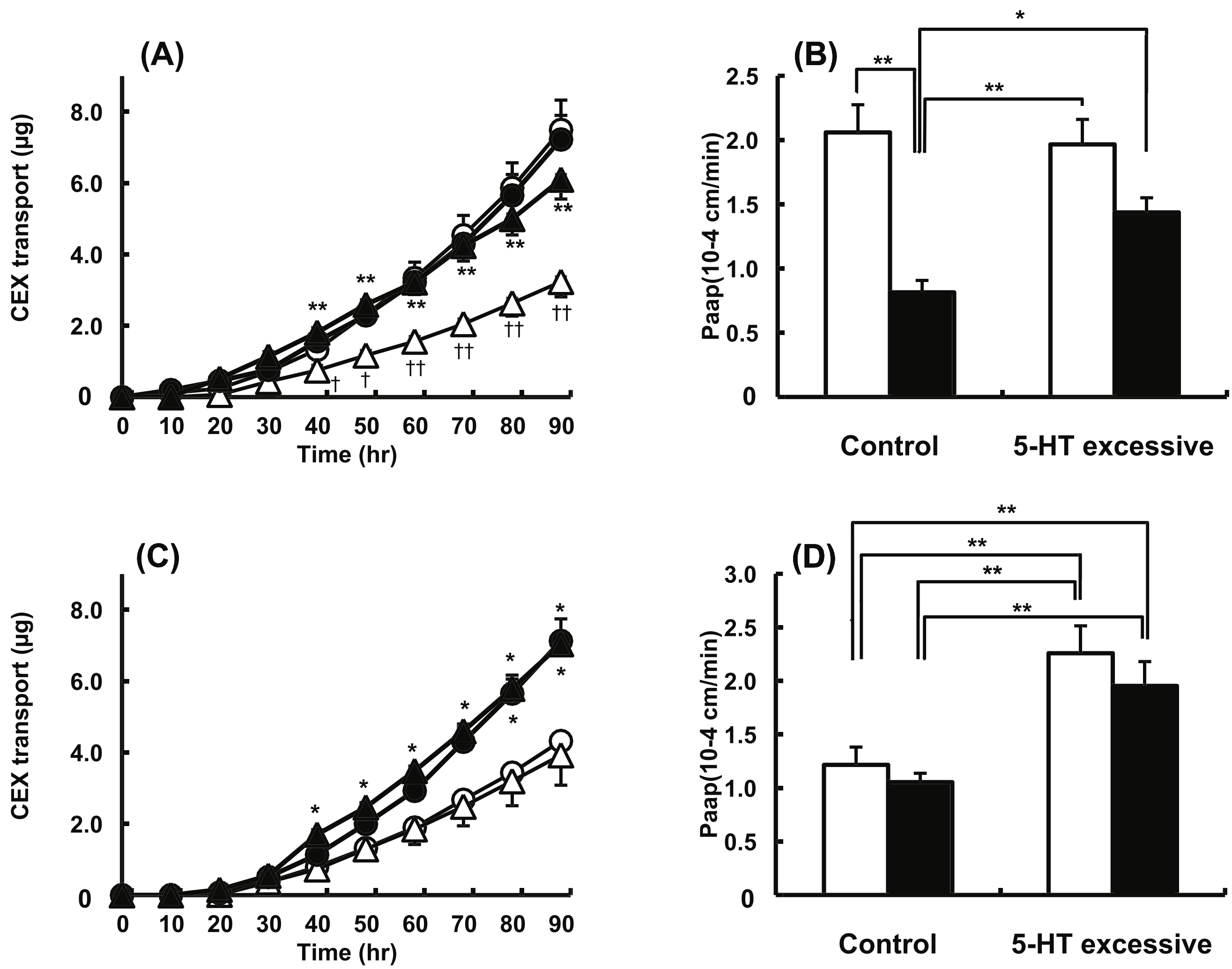


**Fig. 3** Effect of 5-HT-excessive condition on intestinal transport of cephalexin. Cumulative transported amounts (**A**, jejunum; **C**, ileum) and apparent permeability ($P_{app}$) (**B**, jejunum; **D**, ileum) are shown as the mean with S.E. bar of 7 to 14 experiments. **, $p<0.01$; *, $p<0.05$ compared with control rat. ††, $p<0.01$; †, $p<0.05$ compared with corresponding group without Gly-Sar. Keys: (**A**) (**C**)○, control rat; △, control rat with Gly-Sar;●, 5-HT-excessive rat;▲, 5-HT-excessive rat with Gly-Sar. (**B**) (**D**)□, without Gly-Sar;■, with Gly-Sar.

PKC would play a key role for the functional changes in PEPT1 by decreasing the expression level of PEPT1 on brush border membrane under 5-HT-excessive condition. Furthermore, in the in vivo condition, the suppression of $Na^+/H^+$ exchanger (NHE3) function by 5-HT via 5-$HT_4$ [71, 72] would decrease the activity of PEPT1 by reducing the driving force for PEPT1.

Although much of the focus of 5-HT signaling in the small intestine has been related in the role in the motility and inflammation of small intestine [73, 74], a group of RA Cowles have recently reported that the enhanced 5-HT signaling increased the mucosal growth [75] and the small intestinal absorption of glucose and peptide [75] and of carbohydrate and lipid [76]. Since they did not refer to any

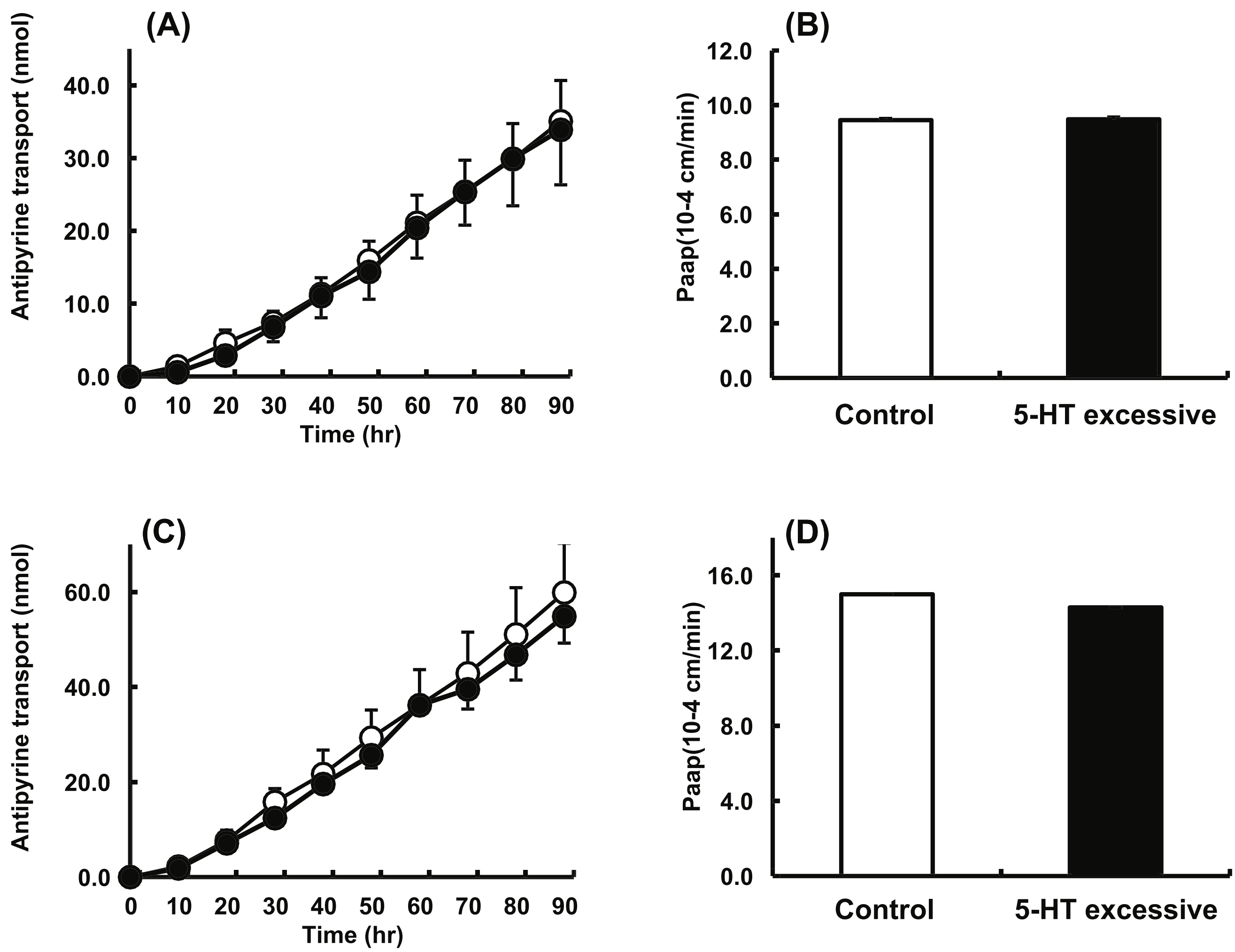


**Fig. 4** No change in intestinal transport of antipyrine under 5-HT-excessive condition. Cumulative transported amounts (**A**, jejunum; **C**, ileum) and apparent permeability ($P_{app}$) (**B**, jejunum; **D**, ileum) are shown as the mean with S.E. bar of 4 to 7 experiments. Keys: (**A**) (**C**), control rat; , 5-HT-excessive rat; (**B**) (**D**), control rat; , 5-HT-excessive rat.

transporters and/or possible change in membrane property such as permeability, the details for their findings remain to be clarified, but it should be very interesting reports suggesting that 5-HT could play an important role in the absorptive function of small intestine.

### Estimation of Gastrointestinal Transit

Figure 3 clearly indicated that the transport of CEX was significantly enhanced in the ileal segment, but not in the jejunum. Since we have already reported that around 50% of CEX absorbed after oral administration would be attributed to the ileal segment [36], the enhanced in vivo absorption of CEX after oral administration shown in Fig. 2(B) could be derived from the absorption from the ileal segment. On the other hand, we have also reported that the change in gastric emptying and GI transit could lead to the change in the segment contributing to the drug absorption after oral administration and, thereby, the change in the extent of bioavailability due to the permeability varied dependent on each segment [77]. Since it is well known that 5-HT plays an important role in GI motility [66], GI transit was evaluated as the mean residence time under 5-HT-excessive condition (Fig. 8). Figure 8 indicated that GI transit time tended to be shorten for the upper regions including the stomach, duodenum and upper jejunum, but the prolonging tendency was observed for the lower segments including the lower jejunum, the upper and lower ileum, meaning that the residence time of CEX was prolonged in the ileum, where the permeability was enhanced. Therefore, the results obtained here also support the larger contribution of ileal segment to the oral absorption of CEX after oral administration under 5-HT-excessive condition.

The shortening GI transit time in the upper segments means the activation of GI motility, which would be via 5-$HT_3$ and/or 5-$HT_4$ [66], but the current results also

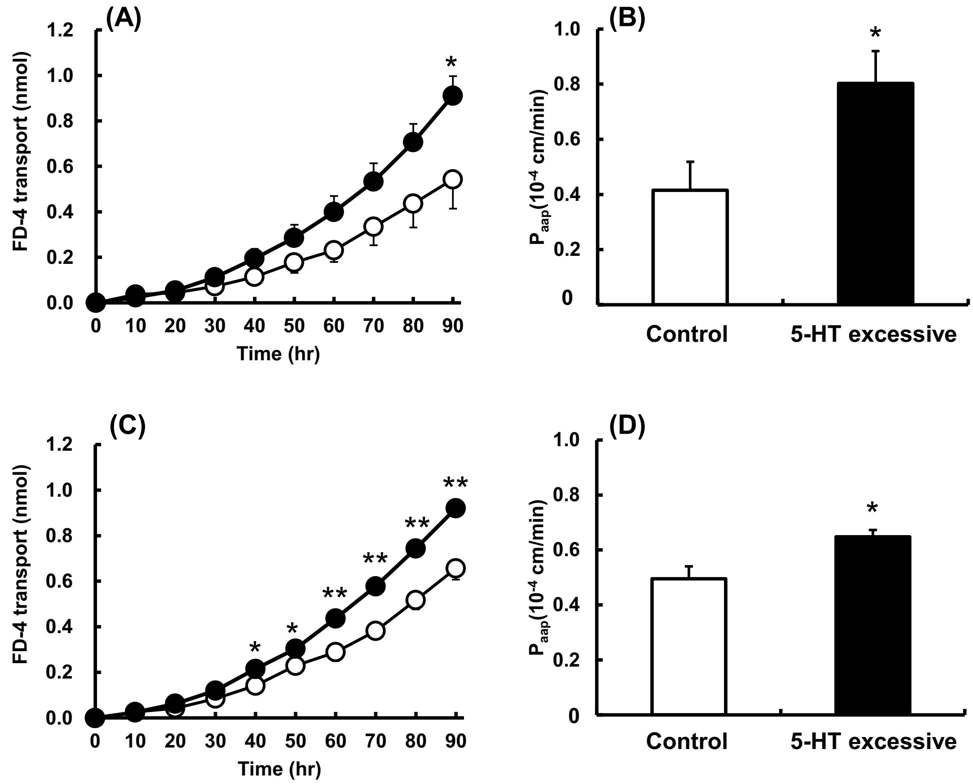


**Fig. 5** Enhancement of intestinal transport of FD-4 under 5-HT-excessive condition. Cumulative transported amounts (**A**, jejunum; **C**, ileum) and apparent permeability ($P_{app}$) (**B**, jejunum; **D**, ileum) are shown as the mean with S.E. bar of 4 to 7 experiments. **, $p<0.01$; *, $p<0.05$ compared with control rat. Keys: (**A**) (**C**)○, control rat;●, 5-HT-excessive rat; (**B**) (**D**)□, control rat;■, 5-HT-excessive rat.

indicated that the activated motility in the upper segments was not propagated to the lower segments (Fig. 8). Under the fasted state, usually, the contraction pattern which begins in the stomach aborally migrates from the duodenum through the ileum, which is called as the migrating motor complex (MMC) [4]. However, the destruction of serotonergic neuron caused the disruption of MMC [66, 78] and it was, furthermore, speculated that the continuous exposure of the mucosa to 5-HT might eventually result in the desensitization of 5-HT receptors and, thereby, decrease the reflex activity [79]. The current result might be also the case with it, although the actual mechanisms still remain to be clarified.

## PEPT2 Expression on Brush Border Membrane of Kidney

Finally, we tried to figure out the reason why CEX was eliminated significantly faster in the elimination phase in

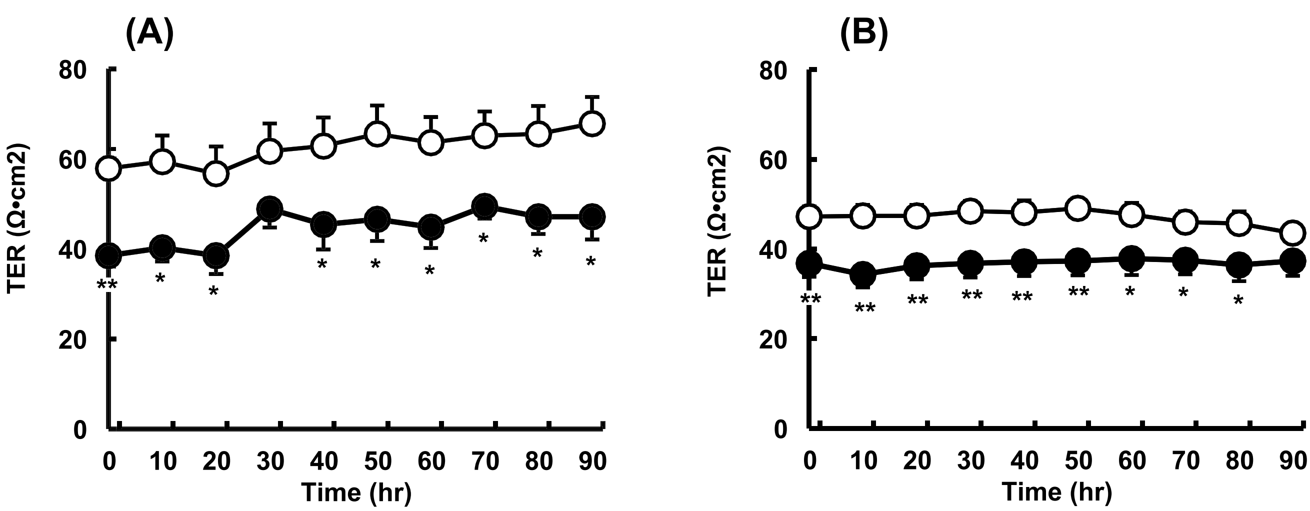


**Fig. 6** Decrease in transmucosal electrical resistance under 5-HT-excessive condition. (**A**) jejunum (**B**) ileum. Results are expressed as the mean with S.E. bar of 4 to 15 experiments. **, $p<0.01$; *, $p<0.05$ compared with control. Keys:○, control rat;●, 5-HT- excessive rat.

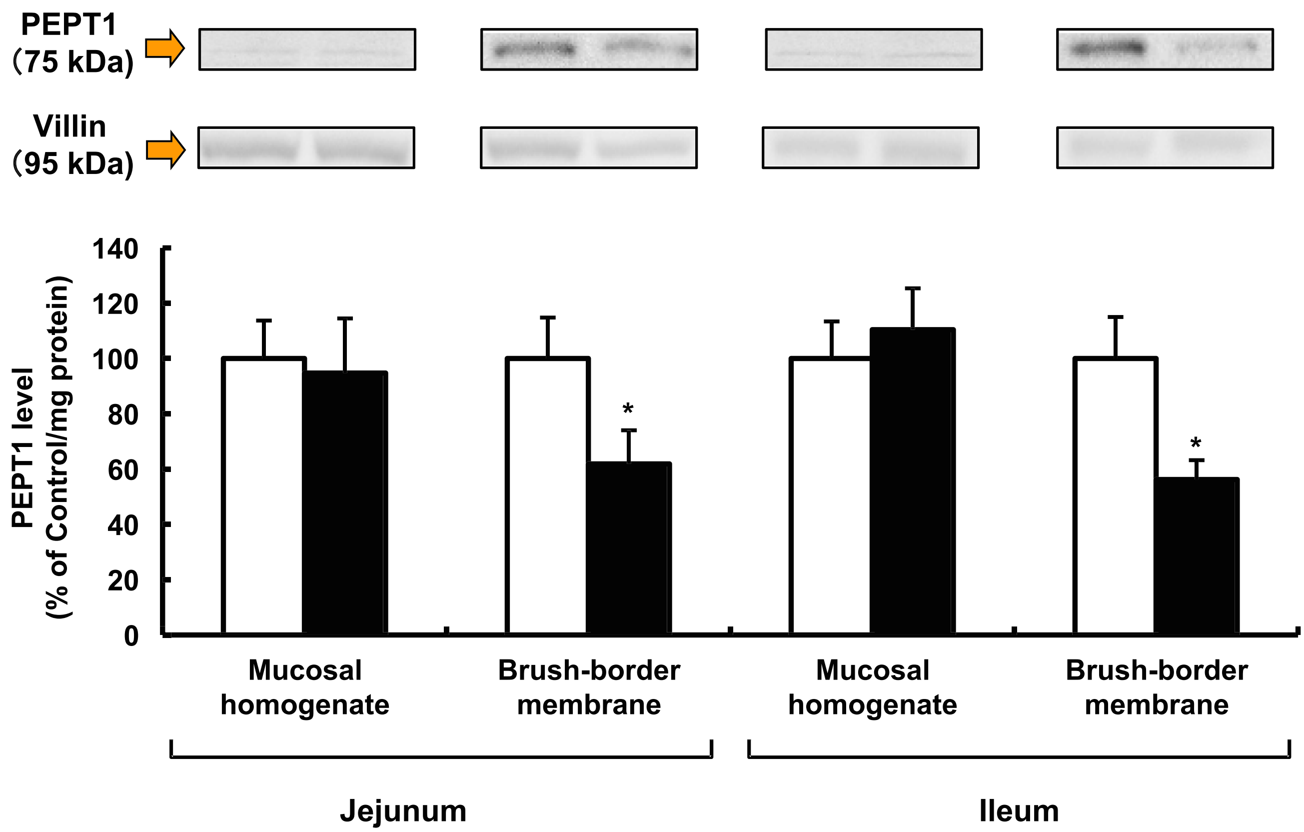


**Fig. 7** Decrease in PEPT1 level on brush border membrane of small intestinal epithelial cells by 5-HT-excessive. Upper panel; typical images of Western blot of PEPT1 and villin. Lower panel; Results are expressed as the mean with S.E. bar of 5 experiments. *, $p<0.05$ compared with control rat. Keys: □, control rat; ■, 5-HT-excessive rat.

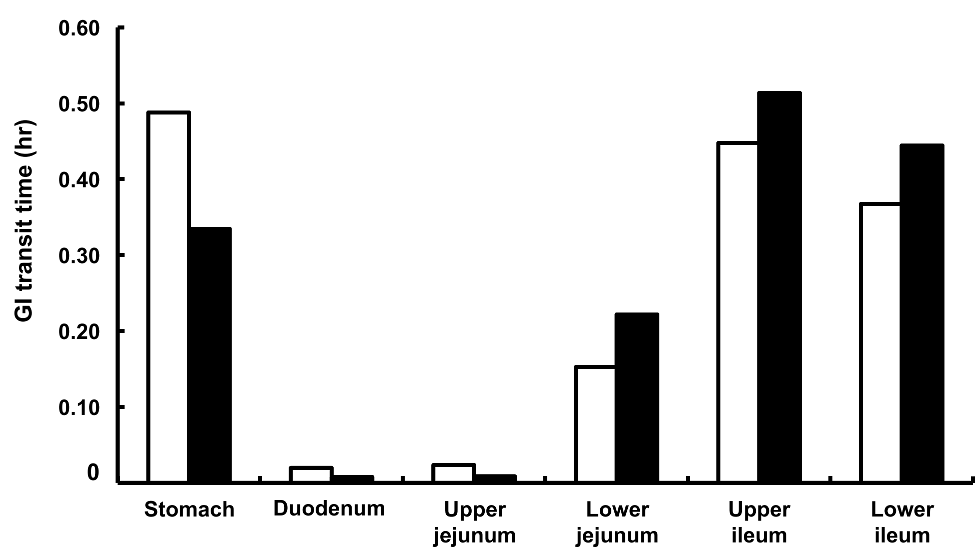


**Fig. 8** Changes in gastrointestinal transit for 5-HT-excessive rats. Mean residence time was calculated as a reciprocal of transit rate constant obtained based on the study of gastrointestinal transit studies of phenol red and/or glass beads. Keys: □, control rat; ■, 5-HT-excessive rat.

5-HT-excessive rats. Since β-lactam antibiotics such as CEX are reabsorbed via PEPT1/PEPT2 from the renal proximal tubules, where PEPT2, having the higher affinity for CEX than PEPT1, is predominantly expressed [80], we focused on PEPT2 and investigated its renal expression. Western blot analysis clearly indicated that the expression level in BBM fraction was significantly decreased in 5-HT-excessive rats (Fig. 9(A) and (B)). Furthermore, since the protein amount normalized by a wet weight of kidney was statistically decreased in 5-HT-excessive rats (2.10 ± 0.14 mg/g

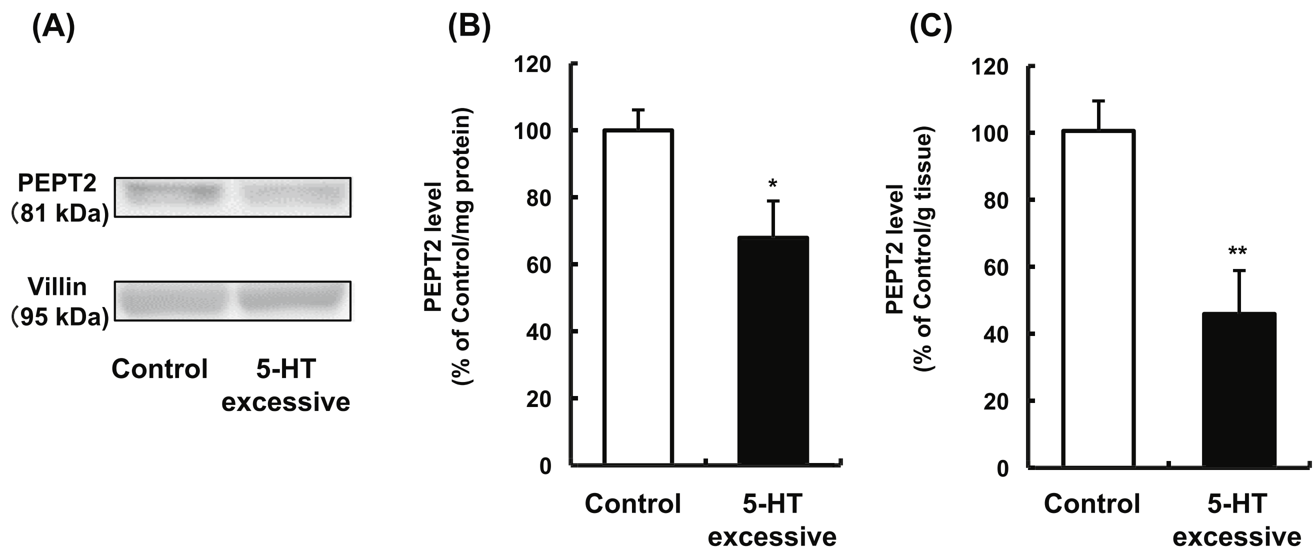


**Fig. 9** 5-HT-excessive condition decreases PEPT2 level on brush border membrane of kidney. (**A**) Typical images of Western blot of PEPT2 and villin. (**B**) (**C**) Results are expressed as the mean with S.E. bar of 6 to 8 experiments. **, $p<0.01$; *, $p<0.05$ compared with control rat. Keys: , control rat; , 5-HT-excessive rat.

kidney; $p<0.01$: control rats, $3.27 \pm 0.17$ mg/g kidney), the expression level normalized by a tissue weight went down to around 46% of control (Fig. 9(C)) and therefore the reabsorptive function of whole kidneys would be even lowered in 5-HT-excessive rats. These results provided that the possible reduction of CEX reabsorption from the renal proximal tubule due to the decreased functional PEPT2 would be a reason for the faster elimination of CEX from plasma (Fig. 2(A)). Since the activity of PEPT2 was decreased by the increase in intracellular $Ca^{2+}$ [81], the trafficking of PEPT2 to the apical membrane might be attenuated so was PEPT1 in the small intestine. Considering the lower protein levels in kidneys observed above, the biosynthesis of PEPT2 itself might also be suppressed. As for the linkage with 5-HT, 5-$HT_1$, 5-$HT_2$ and 5-$HT_7$ are expressed in the kidney [82] and it was reported that PKC was activated through 5-$HT_2$ [83]. Therefore, the activation of PKC through 5-$HT_2$ might have led to the attenuated trafficking of PEPT2 to the apical membrane as discussed above for PEPT1 in the small intestine. However, little data is still available on the regulation of PEPT2 compared with PEPT1 and it was suggested that the regulatory mechanisms for the peptide transporters differed between isoforms and tissues [84], the further study should be needed to clarify the mechanisms behind our findings.

In the current study, we have found the changes in oral absorption and pharmacokinetics of CEX under the 5-HT-excessive condition and tried to figure out the mechanisms behind our findings. Since the level of 5-HT in the brain was also significantly enhanced in the 5-HT-excessive rats we prepared (Fig. 1(B)), some CNS-derived effects might be involved in our findings. It is too complicated to refer to the effects via CNS and we have, thereby, discussed the results obtained in the current study only from the peripheral aspect. Although it is very difficult to differentiate CNS-derived effects from peripheral effects in vivo situation, it has been very important and desired to figure out what would be a peripheral effect or would be derived from CNS in order to understand the effects and/or roles of 5-HT on the function of small intestine as a whole. Furthermore, to evaluate more precisely the intestinal transport of poorly absorbable small molecular drugs via passive diffusion and the possible contribution of putative peptide transporters on basolateral membrane of intestinal epithelial and renal proximal epithelial cells [85] to the intestinal transport and renal excretion, respectively, further studies employing some non-substrates for PEPT1 or PEPT2, i.e., cefotiam (CTM) and cefazolin (CEZ) [86], would be valuable. As it has been reported that CEX is a substrate for multidrug and toxin extrusion proteins (MATEs) [87], the studies with CTM and/or CEZ, which would not be a substrate for MATEs, would also be helpful to clarify the mechanisms of changes in pharmacokinetics of CEX under 5-HT-excessive condition.

In conclusions, higher levels of 5-HT in the small intestine, brain and blood were found in 5-HT-excessive rats, where the oral bioavailability of CEX was significantly increased via the passive diffusion through the expanded paracellular route, but CEX absorption via PEPT1 was attenuated due to the decreased expression of PEPT1 on brush border membrane of small intestinal epithelial cells.

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## Declarations

**Conflict of Interest** The authors declare no competing financial interests.

## References

1. Uesaka T, Young HM, Pachnis V, Enomoto H. Development of the intrinsic and extrinsic innervation of the gut. Dev Biol. 2016;417:158–67.
2. Furness JB. Types of neurons in the enteric nervous system. J Auton Nerv Syst. 2000;81:87–96.
3. Wood JD. Physiology of the enteric nervous system. In: Johnson LR, editor. Physiology of the gastrointestinal tract. New York: Raven; 1994. p. 423–82.
4. Weisbrodt NW. Motility of the small intestine. In: Johnson LR, editor. Gastrointestinal physiology. St. Louis: Mosby; 1997. p. 43–50.
5. Kunze WAA, Furness JB. The enteric nervous system and regulation of intestinal motility. Annu Rev Physiol. 1999;61:117–42.
6. Spencer NJ, Bywater RA. Enteric nerve stimulation evokes a premature colonic migrating motor complex in mouse. Neurogastroenterol Motil. 2002;14:657–65.
7. Nylander O, Hällgren A, Sababi M. Cox inhibition excites enteric nerves that affect motility, alkaline secretion, and permeability in rat duodenum. Am J Phys. 2001;281:G1169–G12178.
8. Chiba T, Bharucha AE, Thomforde GM, Kost LJ, Phillips SF. Model of rapid gastrointestinal transit in dogs: effects of muscarinic antagonists and a nitric oxide synthase inhibitor. Neurogastroenterol Motil. 2002;14:535–41.
9. Hayashi H, Suzuki T, Yamamoto T, Suzuki Y. Cholinergic inhibition of electrogenic sodium absorption in the guinea pig distal colon. Am J Phys. 2003;284:G617–28.
10. Cooke HJ, Reddix RA. Neural regulation of intestinal electrolyte transport. In: Johnson LR, editor. Physiology of the gastrointestinal tract. New York: Raven; 1994. p. 2083–132.
11. Lebrun F, Francois A, Vergnet M, Lebaron-Jacobs L, Bourmelon P, Griffiths NM. Ionizing radiation stimulates muscarinic regulation of rat intestinal mucosal function. Am J Phys. 1998;275:G1333–40.
12. Hörger S, Schultheiß G, Diener M. Segment-specific effects of epinephrine on ion transport in the colon of the rat. Am J Phys. 1998;275:G1367–76.
13. Hayden UL, Carey HV. Neural control of intestinal ion transport and paracellular permeability is altered by nutritional status. Am J Phys. 2000;278:R1589–94.
14. Green BT, Brown DR. Active bicarbonate-dependent secretion evoked by 5-hydroxy-tryptamine in porcine ileal mucosa is mediated by opioid-sensitive enteric neurons. Eur J Pharmacol. 2002;451:185–90.
15. Wang L, Martínez V, Kimura H, Taché Y. 5-Hydroxytryptophan activates colonic myenteric neurons and propulsive motor function through 5-HT4 receptors in conscious mice. Am J Phys. 2007;292:G419–28.
16. De Giorgio R, Barbara G, Furness JB, Tonini M. Novel therapeutic targets for enteric nervous system disorders. Trends Pharmacol Sci. 2007;28:473–81.
17. Li Z, Chalazonitis A, Huang Y-Y, Mann JJ, Margolis KG, Yang QM, Kim DO, Côté F, Mallet J, Gershon MD. Essential roles of enteric neuronal serotonin in gastrointestinal motility and the development/survival of enteric dopaminergic neurons. J Neurosci. 2011;31:8998–9009.
18. Kadowaki M, Nagakura Y, Tomoi M, Mori J, Kohsaka M. Effect of FK1052, a potent 5-hydroxytryptamine$_3$ and potent 5-hydroxytryptamine$_4$ receptor dual antagonist, on colonic function in vivo. J Pharmacol Exp Ther. 1993;66:74–80.
19. Shajib MS, Khan WI. The role of serotonin and its receptors in activation of immune responses and inflammation. Acta Physiol. 2015;213:561–74.
20. Walsh KT, Zemper AE. The enteric nervous system for epithelial researchers: basic anatomy, techniques, and interactions with the epithelium. Cell Mol Gastroenterol Hepatol. 2019;8:369–78.
21. Atkinson W, Lockhart S, Whorwell PJ, Keevil B, Houghton LA. Altered 5-hydroxytryptamine signaling in patients with constipation- and diarrhea-predominant irritable bowel syndrome. Gastroenterol. 2006;130:34–43.
22. Villanacci V, Bassotti G, Nascimbeni R, Antonelli E, Cadei M, Fisogni S, Salerni B, Geboes K. Enteric nervous system abnormalities in inflammatory bowel diseases. Neurogastroenterol Motil. 2008;20:1009–16.
23. Mogilevski T, Burgell R, Aziz Q, Gibson PR. Review article: the role of the autonomic nervous system in the pathogenesis and therapy of IBD. Aliment Pharmacol Ther. 2019;50:720–37.
24. Spiller R. Serotonin and GI clinical disorders. Neuropharmacol. 2008;55:1072–80.
25. Magro F, Vieira-Coelho MA, Fraga S, Serrao MP, Veloso FT, Ribeiro T, Soares-da-Silva P. Impaired synthesis or cellular storage of norepinephrine, dopamine, and 5-hydroxytryptamine in human inflammatory bowel disease. Dig Dis Sic. 2002;47:216–24.
26. Haugen M, Dammen R, Svejda B, Gustafsson BI, Pfragner R, Modlin I, Kidd M. Differential signal pathway activation and 5-HT function: the role of gut enterochromaffin cells as oxygen sensors. Am J Physiol Gastrointest Liver Physiol. 2012;303:G1164–73.
27. Neunlist M, Toumi F, Oreschkova T, Denis M, Leborgne J, Laboisse CL, Galmiche JP, Jarry A. Human ENS regulates the intestinal epithelial barrier permeability and a tight junction-associated protein ZO-1 via VIPergic pathways. Am J Physiol Gastrointest Liver Physiol. 2003;285:G1028–36.
28. Higaki K, Sone M, Ogawara K, Kimura T. Regulation of drug absorption from small intestine by enteric nervous system I: a poorly absorbable drug via passive diffusion. Drug Metab Pharmacokin. 2004;19:198–205.
29. Hiraoka H, Kimura N, Furukawa Y, Ogawara K, Kimura T, Higaki K. Up-regulation of P-glycoprotein expression in small intestine under chronic serotonin-depleted conditions in rats. J Pharmacol Exp Ther. 2005;312:248–55.
30. Kimoto T, Takanashi M, Mukai H, Ogawara K, Kimura T, Higaki K. Effect of adrenergic stimulation on drug absorption via passive diffusion in Caco-2 cells. Int J Pharm. 2009;368:31–6.
31. Mukai H, Takanashi M, Ogawara K, Maruyama M, Higaki K. Possible regulation of P-glycoprotein function by adrenergic agonists in a vascular-luminal perfused preparation of small intestine. J Pharm Sci. 2021;110:3889–95.
32. Stouch TR, Gudmundsson O. Progress in understanding the structure-activity relationships of P-glycoprotein. Adv Drug Deliv Rev. 2002;54:315–28.
33. Nishijima K, Yoshino T, Ishiguro T. Risperidone counteracts lethality in an animal model of the serotonin -excessive. Psychopharmacol. 2000;150:9–14.

34. Squires LN, Talbot KN, Rubakhin SS, Sweedler JV. Serotonin catabolism in the central and enteric nervous systems of rats upon induction of serotonin -excessive. J Neurochem. 2007;103:174–80.
35. Walter DJ, Peter J-U, Bashammakh S, Hörtnagl H, Voits M, Fink H, Bader M. Synthesis of serotonin by a second tryptophan hytdroxylase isoform. Science. 2003;299:76.
36. Hironaka T, Itokawa S, Ogawara K, Higaki K, Kimura T. Quantitative evaluation of PEPT1 contribution to oral absorption of cephalexin in rats. Pharm Res. 2009;26:40–50.
37. Pan X, Terada T, Irie M, Saito H, Inui K. Diurnal rhythm of $H^+$-peptide cotransporter in rat small intestine. Am J Phys. 2002;283:G57–64.
38. Pan X, Terada T, Okuda M, Inui K. The diurnal rhythm of the intestinal transporters SGLT1 and PEPT1 is regulated by the feeding conditions in rats. J Nutr. 2004;134:2211–5.
39. Pan X, Terada T, Okuda M, Inui K. Altered diurnal rhythm of intestinal peptide transporter by fasting and its effects on the pharmacokinetics of ceftibuten. J Pharmacol Exp Ther. 2003;307:626–32.
40. Lakshmana MK, Raju TR. An isocratic assay for norepinephrine, dopamine, and 5-hydroxytryptamine using their native fluorescence by high-performance liquid chromatography with fluorescence detection in discrete brain areas of rat. Anal Biochem. 1997;15:166–70.
41. Kessler M, Acuto O, Storelli C, Murer H, Müller M, Semenza G. A modified procedure for the rapid preparation of efficiently transporting vesicle from small intestinal brush border membranes. Biochim Biophys Acta. 1978;506:136–54.
42. Wilfong RF, Neville DM Jr. The isolation of a brush border membrane fraction from rat kidney. J Biol Chem. 1970;245:6106–13.
43. Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature. 1970;227:680–5.
44. Kadono K, Yokoe J, Ogawara K, Higaki K, Kimura T. Analysis and prediction of absorption behavior for theophylline orally administered as powders based on gastrointestinal-transit-absorption (GITA) model. Drug Metab Pharmacokinet. 2002;17:307–15.
45. Haruta S, Kawai K, Jinnouchi S, Ogawara K, Higaki K, Tamura S, Arimori K, Kimura T. Evaluation of absorption kinetics of orally administered theophylline in rats based on gastrointestinal transit monitoring by gamma scintigraphy. J Pharm Sci. 2001;90:464–73.
46. Davis SS, Hardy JG, Fara JW. Transit of pharmaceutical dosage forms through the small intestine. Gut. 1986;27:886–92.
47. Abrahamsson B, Alpsten M, Hugosson M, Jonsson ULFE, Sundgren M, Svenheden A, Tölli J. Absorption, gastrointestinal transit, and tablet erosion of felodipine extended-release (ER) tablets. Pharm Res. 1993;10:709–14.
48. Peh KK, Yuen KH. Indirect gastrointestinal transit monitoring and absorption of theophylline. Int J Pharm. 1996;139:95–103.
49. Tuleu C, Andrieux C, Boy P, Chaumeil JC. Gastrointestinal transit of pellets in rats: effect of size and density. Int J Pharm. 1999;180:123–31.
50. Devereux JE, Newton JM, Short MB. The influence of density on the gastrointestinal transit of pellets. J Pharm Pharmacol. 1990;42:500–1.
51. Hinder RA, Kelly KA. Canine gastric emptying of solids and liquids. Am J Phys. 1977;233:E335–40.
52. Camilleri M, Malagelada JR, Brown ML, Becker G. Relation between antral motility and gastric emptying of solids and liquids in humans. Am J Phys. 1985;249:G580–5.
53. Sawamoto T, Haruta S, Kurosaki Y, Higaki K, Kimura T. Prediction of the plasma concentration profiles of orally administered drugs in rats on the basis of gastrointestinal transit kinetics and absorbability. J Pharm Pharmacol. 1997;49:450–7.
54. Haruta S, Kawai K, Nishii R, Jinnouchi S, Ogawara K, Higaki K, Tamura S, Arimori K, Kimura T. Prediction of plasma concentration – time curve of orally administered theophylline based on a scintigraphic monitoring of gastrointestinal transit in human volunteers. Int J Pharm. 2002;233:179–90.
55. Fujioka Y, Metsugi Y, Ogawara K, Higaki K, Kimura T. Evaluation of in vivo dissolution behavior and GI transit of griseofulvin, a BCS class II drug. Int J Pharm. 2008;352:36–43.
56. Yamaoka K, Tanigawara Y, Tanaka H, Uno T. A pharmacokinetic analysis program (MULTI) for microcomputer. J Pharmacobio-Dyn. 1981;4:879–85.
57. Gill RK, Pant N, Saksena S, Singla A, Nazir TM, Vohwinkel L, Turner JR, Goldstein J, Alrefal WA, Dudeja PK. Function, expression, and characterization of the serotonin transporter in the native human intestine. Am J Physiol Gastrointest Liver Physiol. 2008;294:G254–62.
58. Nakatani Y, Sato-Suzuki I, Tsujino N, Nakasato A, Seki Y, Fumoto M, Arita H. Augmented brain 5-HT crosses the blood-brain barrier through the 5-HT transporter in rat. Eur J Neurosci. 2008;27:2466–72.
59. Wakayama K, Ohtsuki S, Takanaga H, Hosoya K, Terasaki T. Localization of norepinephrine and serotonin transporter in mouse brain capillary endothelial cells. Neurosci Res. 2002;44:173–80.
60. Chryssafidis P, Tsekouras AA, Macheras P. Re-writing oral pharmacokinetics using physiologically based finite time pharmacokinetic (PBFTPK) models. Pharm Res. 2022;39:691–701.
61. Turner JR, Rill BK, Carlson SL, Carnes D, Kerner R, Mrsny RJ, MADARA JL. Physiological regulation of epithelial tight junctions is associated with myosin light-chain phosphorylation. Am J Phys Cell Phys. 1997;273:C1378–85.
62. Shen L, Black ED, Witkowski ED, Lencer WI, Guerriero V, Schneeberger EE, Turner JR. Myosin light chain phosphorylation regulates barrier function by remodeling tight junction structure. J Cell Sci. 2006;119:2095–106.
63. Beattie DT, Smith JAM. Serotonin pharmacology in the gastrointestinal tract: a review. Naunyn Schmiedeberg's Arch Pharmacol. 2008;377:181–203.
64. Iceta R, Mesonero JE, Aramayona JJ, Alcalde AI. Expression of 5-$HT_{1A}$ and 5-$HT_7$ receptors in Caco-2 cells and their role in the regulation of serotonin transporter activity. J Physiol Pharmacol. 2009;60:147–64.
65. Assender JW, Irenius E, Fredholm BB. 5-Hydroxytryptamine, angiotensin and bradykinin transiently increase intracellular calcium concentrations and PKC-α activity, but do not induce mitogenesis in human vascular smooth muscle cells. Acta Physiol Scand. 1997;160:207–17.
66. Spiller R. Serotonergic modulating drugs for functional gastrointestinal diseases. Br J Clin Pharmacol. 2002;54:11–20.
67. Tuo B-G, Sellers Z, Paulus P, Barrett KE, Isenberg JI. 5-HT induces duodenal mucosal bicarbonate secretion via cAMP- and $Ca^{2+}$-dependent signaling pathways and 5-$HT_4$ receptors in mice. Am J Physiol Gastrointest Liver Physiol. 2004;286:G444–51.
68. Mace OJ, Lister N, Morgan E, Shepherd E, Affleck J, Helliwell P, Bronk JR, Kellett GL, Meredith D, Boyd R, Pieri M, Bailey PD, Pettcrew R, Foley D. An energy supply network of nutrient absorption coordinated by calcium and T1R taste receptors in rat small intestine. J Physiol. 2009;587:195–210.
69. Lee VHL. Membrane transporters. Eur J Pharm Sci. 2000;11(sup2):S41–50.
70. Berlioz F, Maoriet J-J, Paris H, Laburthe M, Farinotti R, Rozé C. α2-adrenergic receptors stimulate oligopeptide transport in a huma intestinal cell line. J Pharmacol Exp Ther. 2000;294:466–72.
71. Gill RK, Saksena S, Tyagi S, Alrefai WA, Malakooti J, Turner SZ, Ramaswamy JR, K, Dudeja PK. Serotonin inhibits $Na^+/H^+$

exchange activity via 5-HT4 receptors and activation of PKCα in human intestinal epithelial cells. Gastroenterol. 2005;128:962–74.
72. Amin MR, Ghannad L, Othman A, Gill RK, Dudeja PK, Ramaswamy K, Malakooti J. Transcriptional regulation of the human $Na^+/H^+$ exchanger NHE3 by serotonin in intestinal epithelial cells. Biochem Biophys Res Commun. 2009;382:620–5.
73. Gershon MD. 5-Hydroxytryptamine (serotonin) in the gastrointestinal tract. Curr Opin Endocrinol Diabetes Obes. 2013;20:14–21.
74. Spohn SN, Mawe GM. Non-conventional features of peripheral serotonin signaling – the gut and beyond. Nat Rev Gastroenterol Hepatol. 2017;14:412–20.
75. Greig CJ, Zhang L, Cowles RA. Potentiated serotonin signaling in serotonin re-uptake transporter knockout mice increases enterocyte mass and small intestinal absorptive function. Phys Rep. 2019;7:e14278.
76. Park CJ, Amrenia SJ, Shaughnessy MP, Greig CJ, Cowles RA. Potentiation of serotonin signaling leads to increased carbohydrate and lipid absorption in the murine small intestine. J Pediatr Surg. 2019;54:1245–9.
77. Haruta S, Iwasaki N, Ogawara K, Higaki K, Kimura T. Absorption behavior of orally administered drugs in rats treated with propantheline. J Pharm Sci. 1998;87:1081–5.
78. Pineiro-Carrero VM, Clench MH, Davis RH, Andres JM, Franzini DA, Mathias JR. Intestinal motility changes in rats after enteric serotonergic neuron destruction. Am J Physiol Gastrointest Liver Physiol. 1991;260:G232–9.
79. Coates MD, Mahoney CR, Linden DR, Sampson JE, Chen J, Blaszyk H, Crowell MD, Sharkey KA, Gershon MD, Mawe GM, Moses PL. Molecular defects in mucosal serotonin content and decreased serotonin reuptake transporter in ulcerative colitis and irritable bowel syndrome. Gastroenterol. 2004;126:1657–64.
80. Nakanishi T, Fukushi A, Sato M, Yoshifuji M, Gose T, Shirasaka Y, Ohe K, Kobayashi M, Kawai K, Tamai I. Functional characterization of apical transporters expressed in rat proximal tubular cells (PTCs) in primary culture. Mol Pharm. 2011;8:2142–50.
81. Wenzel U, Diehl D, Herget M, Kuntz S, Daniel H. Regulation of the high-affinity $H^+$/peptide cotransporter in renal LLC-$PK_1$ cells. J Cell Physiol. 1999;178:341–8.
82. Gibbs WS, Collier JB, Morris M, Beeson CC, Megyesi J, Schnellmann RG. 5-$HT_{1F}$ receptor regulates mitochondrial homeostasis and its loss potentiates acute kidney injury and impairs renal recovery. Am J Physiol Ren Physiol. 2018;315:F1119–28.
83. Hamasaki Y, Doi K, Maeda-Mamiya R, Ogasawara E, Katagiri D, Tanaka T, Yamamoto T, Sugaya T, Nangaku M, Noiri E. A 5-hydroxytryptamine receptor antagonist, sarpogrelate, reduces renal tubulointerstitial fibrosis by suppressing PAI-1. Am J Physiol Ren Physiol. 2013;305:F1796–803.
84. Terada T, Inui K. Gene expression and regulation of drug transporters in the intestine and kidney. Biochem Pharmacol. 2007;73:440–9.
85. Brandsch M, Knütter I, Bosse-Doenecke E. Pharmaceutical and pharmacological importance of peptide transporters. J Pharm Pharmacol. 2008;60:543–85.
86. Shimizu R, Sukegawa T, Tsuda Y, Itoh T. Quantitative prediction of oral absorption of PEPT1 substrates based on in vitro uptake into Caco-2 cells. Int J Pharm. 2008;354:104–10.
87. Nies AT, Damme K, Kruck S, Schaeffeler E, Schwab M. Structure and function of multidrug and toxin extrusion proteins (MATEs) and their relevance to drug therapy and personalized medicine. Arch Toxicol. 2016;90:1555–84.