JPP
Journal of
Pharmacy and Pharmacology

JPP 2006, 58: 195–200
© 2006 The Authors
Received June 27, 2005
Accepted November 3, 2005
DOI 10.1211/jpp.58.2.0006
ISSN 0022-3573

Activation of P2Y receptor enhances high-molecular compound absorption from rat ileum

Natsumi Kinoshita, Tomoko Takahashi, Shoko Tada,
Kazumasa Shinozuka, Nobuyasu Mizuno and Koichi Takahashi

Abstract

While there are no reports concerning the effects of extracellular nucleotides on the intestinal absorption of drugs, it is well known that extracellular nucleotides are important regulators of intestinal epithelial ion transport. This report using fluorescein isothiocyanate dextran 4000 (FD-4) as the model compound is the first to investigate the effects of purine nucleotides on absorption of poorly absorbed drugs from intestine. ATP enhanced the absorption of FD-4 from rat ileum in a concentration-dependent manner. ADP also enhanced the absorption of FD-4. Other purine nucleotides (adenosine, AMP, UTP and UDP) did not show an absorption-enhancing effect. The absorption-enhancing effect by ATP was inhibited by suramin and pyridoxalphosphate-6-azophenyl-2′,4′-disulfonate (PPADS), which are known P2 receptor antagonists. Additionally, 2-methylthio ATP (a P2Y receptor agonist) enhanced the absorption of FD-4, but α,β-methylene ATP (a P2X receptor agonist) did not. These findings suggest that activation of the P2Y receptor may improve the absorption of water-soluble and high-molecular compounds from the ileum.

Introduction

Nucleotides are ubiquitous signalling molecules that induce a wide spectrum of biological responses (Communi & Boeynaems 1997). Extracellular nucleotides are generally thought to regulate a wide range of tissue functions via direct stimulation of membrane P2 receptors (Ralevic & Burnstock 1998). P2 receptors are subdivided into two families, P2X and P2Y receptors (Abbracchio & Burnstock 1994; Fredholm et al 1994). P2X receptors are ATP-gated ion channels and P2Y receptors belong to the superfamily of G-protein-coupled receptors (Benham & Tsien 1987; Dubyak 1991). To date, at least seven P2X receptors (P2X1 to P2X7) and seven P2Y receptors (P2Y1, P2Y2, P2Y4, P2Y6, P2Y11, P2Y12 and P2Y13) have been identified in various animal species (Ralevic & Burnstock 1998). P2X and P2Y receptors are found in a variety of epithelial tissues and have been shown to function as important regulators of ion transport (Leipziger 2003). P2 receptors in gastrointestinal epithelial cells also regulate ion transport (Inoue et al 1997). However, there have been no reports or investigations on the effects of P2 receptors on the intestinal absorption of drugs, especially water-soluble macromolecules.

In the intestine, drug absorption is classified into transcellular and paracellular pathways. The lipophilic compounds are mainly transported through the former, and water-soluble or ionized drugs permeate through the latter. However, the absorption of water-soluble high-molecular compounds is frequently restricted by their poor intestinal permeability through the paracellular pathway. This is because a tight junction exists as a barrier in intestinal epithelium and regulates the permeability of these compounds (Madara 1989; Anderson & Van Itallie 1995). Thus, it is important to study how absorption can be improved. Several compounds, including protease inhibitors, surfactants, bile salts, chelating agents and fatty acids, have been developed to increase the intestinal absorption of polar drugs (Lee & Yamamoto 1990; Tomita et al 1996; Sakai et al 1999). Fatty acids are reported to be able to open the tight junctions through an increase in the intracellular calcium level (Lindmark et al 1998; Hayashi et al 1999; Kimura et al 2001).

In this study, the effect of ATP and other purine nucleotides on the absorption of water-soluble high-molecular compounds was investigated by an in-situ loop and everted sac

Departments of Pharmaceutics and Pharmacology, School of Pharmaceutical Sciences, Mukogawa Women's University, 11-68, Koshien, Kyuban-cho, Nishinomiya 663-8179, Japan

Natsumi Kinoshita, Tomoko Takahashi, Shoko Tada, Kazumasa Shinozuka, Nobuyasu Mizuno, Koichi Takahashi

Correspondence: Koichi Takahashi, Department of Pharmaceutics, School of Pharmaceutical Sciences, Mukogawa Women's University, 11-68, Koshien, Kyuban-cho, Nishinomiya 663-8179, Japan. E-mail: koichi@mwu.mukogawa-u.ac.jp

method. Fluorescein isothiocyanate dextran 4000 (FD-4), which is known to have poor absorption, was chosen as the model compound. We also examined the degradation of ATP in intestinal lumen.

Materials and Methods

Materials

FD-4, ATP disodium salt, 2-methylthio ATP (2-MeSATP), α,β-methylene ATP (α,β-meATP) and pyridoxalphosphate-6-azo-phenyl-2′,4′ disulfonic acid (PPADS) were purchased from Sigma Chemical Co. (St Louis, MO, USA). Sodium laurate (C12), sodium deoxycholate (DC-Na) and suramin sodium were obtained from Wako Pure Chemicals Ltd (Osaka, Japan). All other chemicals were of the finest reagent grade available.

Animals

Male Wistar/ST rats (Japan SLC, Hamamatsu, Japan), weighing 220–290 g, were used in accordance with the Guidelines for Animal Experimentation of the Mukogawa Women's University, which are based on the Guidelines for Animal Experimentation of the Japanese Association for Laboratory Animal Science.

In-situ loop experiments

Absorption studies of FD-4 from the in-situ loop of rat ileum were performed by the method described previously (Yamamoto et al 1996; Numata et al 2000). Briefly, the rats were allowed to fast for 18–20 h before the experiments, with water freely available. After the rats had been anesthetized with ethyl carbamate (1 g kg^{-1}), the ileum was exposed by a middle abdominal incision. The lumen of ileum was washed with phosphate buffered saline (PBS, 20 mL) twice and a loop, 5 cm in length, was prepared by closing both ends with glass tubes attached to a syringe. Test compounds were dissolved with PBS and their pH adjusted to 7.0 with 1.0 M NaOH. They were then administered into the loop at a dose of 8.8 mg kg^{-1}. After administration, blood samples from the jugular vein were collected periodically. The plasma concentration of FD-4 was measured using a fluorescence spectrophotometer. The peak concentration (C_{max}) and time to reach C_{max} (T_{max}) were determined directly from the concentration–time profiles. The area under the plasma concentration–time curve (AUC) was calculated by the trapezoidal method from time zero to the final sampling time.

In-vitro everted sacs experiments

Everted sacs of rat ileum were prepared using a method described before (Kimura et al 1997; Barthe et al 1998). After decapitation of the rat and laparotomy, the ileum was taken above the caecum, washed with isotonic saline and everted. It was cut into 5-cm segments that were ligated at one end, filled on the serosal side with 0.5 mL modified Krebs–Henseleit bicarbonate buffer (KHBB contained in mM: NaCl 119, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25, glucose 11) and tightly ligated to create a gut sac. Immediately, this everted sac was placed in 20 mL of KHBB incubation solution containing 1.1 mg mL^{-1} FD-4 with or without ATP. In some experiments suramin was added in the mucosal solution. The solution was gassed by 95% O_2/5% CO_2 and maintained at 37°C throughout the experiment. After 30 min the sac was removed, washed three times in isotonic saline and blotted dry. The sac was cut open and the serosal fluid drained into a small tube.

Reversibility study

The recovery of the intestine after exposure to the ATP was studied by measuring the plasma concentration of FD-4 after pretreatment with ATP. In this study, initially ATP solutions were administered into the loop on the rat ileum and the loop was washed with 15 mL PBS after 60 min. Subsequently, FD-4 was administered into the loop and the plasma concentration of FD-4 was assayed by the same method used for the absorption study.

FD-4 measurement

Three millilitres of PBS was added to each 100 μL of plasma sample or serosal fluid. The concentration of FD-4 was measured with a fluorescence spectrophotometer (Jasco FP-750, Tokyo, Japan) at excitation and emission wavelengths of 495 and 515 nm, respectively. A standard calibration curve for FD-4 (0.1–5.0 μg mL^{-1}) was constructed using a known amount of FD-4. Correlation coefficients greater than 0.999 were obtained.

ATP degradation experiment

The ATP degradation experiment in rat ileum was performed by the following method. Male Wistar/ST rats, weighing 220–290 g, were used and a loop was prepared in the ileum using the same method as described in the above absorption study. ATP was dissolved in PBS and the solution of ATP was administered into the loop at a dose of 8.8 mg kg^{-1}. After the experiment, intestinal fluids were collected by washing the area with 15 mL PBS. The concentrations of adenine nucleotides in these samples were measured by the following method.

ATP measurement

Samples (100 μL) were deproteinized with methanol (1 mL) and centrifuged. Supernatants (1 mL) were collected and evaporated before being dissolved with 10 mM KH_2PO_4 buffer (pH = 7.0). The ATP concentration was subsequently analysed by reverse-phase HPLC. The HPLC system consisted of a pump (LC-10ADvp, Shimadzu, Kyoto, Japan), a UV detector (SPD-10Avp, Shimadzu, Kyoto, Japan) and an integrator (SCL-10Avp, Shimadzu, Kyoto, Japan). Separation of nucleotides was performed on a 4.6×150 mm column packed with CrestPak C18S (Jasco Co., Tokyo, Japan) using a 10 mM KH_2PO_4 buffer (pH = 7.0) containing 10.0% (v/v) methanol and 1.95 mM of the ion-pairing agent tetrabutylammonium hydrogen

sulfate as eluate and a flow velocity of $1\,mL\,min^{-1}$ at ambient temperature. Mobile phases were filtered through $0.45\,\mu m$ filters (Millipore) before use. Sample volumes of $50\,\mu L$ were injected onto the column and ATP was detected as the absorbance at 254 nm. ATP concentrations for each of the samples were calculated by a standard calibration curve for ATP ($5.0–100.0\,\mu g\,mL^{-1}$). Correlation coefficients were obtained greater than 0.998. Additionally, the reproducibility of quantification was guaranteed. The inter-day and intra-day coefficients of variation were 1.20 and 0.97%, respectively.

Statistical evaluations

All results were expressed as mean ± s.e.m. Statistical significance was determined by analysis of variance with the Bonferroni method used to compare individual data for a significant *F* value. Differences were considered significant when the calculated *P* value was < 0.05.

Results

Figure 1 shows the time course of FD-4 concentration in plasma after its administration into the ileum with or without ATP. While FD-4 only was poorly absorbed from the ileum, it was rapidly absorbed when co-administered with ATP. The absorption of FD-4 increased in an ATP concentration-dependent manner. The pharmacokinetic parameters of FD-4 are listed in Table 1. The values of the maximum concentration (C_{max}) and AUC at 10 mM ATP were about five and four times higher than the values for samples where ATP was absent, respectively. To evaluate the mucosal cytotoxicity of ATP, the reversibility of the enhancement effect by ATP was studied by measuring the plasma concentration of FD-4 after pretreatment with 0 to 10 mM ATP. The concentration of FD-4 after pretreatment with various concentrations of ATP decreased compared with the co-administration of FD-4 and the same concentrations of ATP.

To further investigate whether or not the ATP-induced enhancement effect was mediated by stimulating the P2 receptor, the effect was assessed in the presence of suramin and PPADS, both of which are P2 receptor antagonists. As shown in Figure 2, the ATP (10 mM)-induced effect was significantly inhibited by the co-administration of 2 mM suramin or 2 mM PPADS. Figure 3 shows the effects of 2-MeSATP (P2Y receptor agonist) and α,β-meATP (P2X receptor agonist) on FD-4 absorption. Absorption of FD-4 was enhanced

Table 1 FD-4 pharmacokinetic parameters after FD-4 administration into rat ileum in the absence or presence of 5 or 10 mM ATP

ATP (mM)	C_{max} ($\mu g\,mL^{-1}$)	T_{max} (min)	AUC ($\mu g\,mL^{-1}$/min)
0	0.21 ± 0.07	142.5 ± 44.8	24.8 ± 2.4
5	0.61 ± 0.07	100.0 ± 70.0	73.3 ± 6.9[a]
10	0.98 ± 0.19[a]	30.0 ± 0.0[c]	94.5 ± 11.1[b]

Each value represents the mean ± s.e.m. (n = 4–6, respectively). [a]$P < 0.05$ compared with 0 mM ATP. [b]$P < 0.01$ compared with 0 mM ATP. [c]The standard error of T_{max} was not observed.

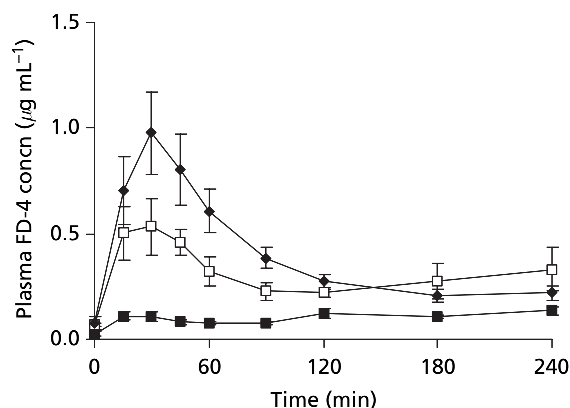


Figure 1 Time course of plasma FD-4 concentrations after FD-4 administration into rat ileum in the absence (■) or presence of 5 mM (□) or 10 mM (♦) ATP. Each point and the vertical bars represent the mean ± s.e.m. (n = 4–6, respectively).

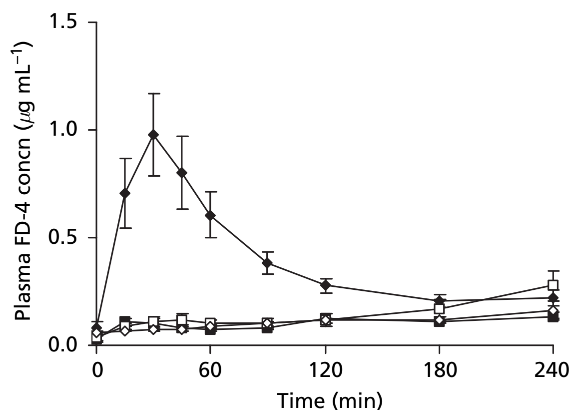


Figure 2 Effect of suramin and PPADS on ATP-induced enhancement of FD-4 absorption in rat ileum. Symbols: control (■); 10 mM ATP (♦); 10 mM ATP + 2 mM suramin (□); 10 mM ATP + 2 mM PPADS (◇). Each point and the vertical bars represent the mean ± s.e.m. (n = 4–6, respectively).

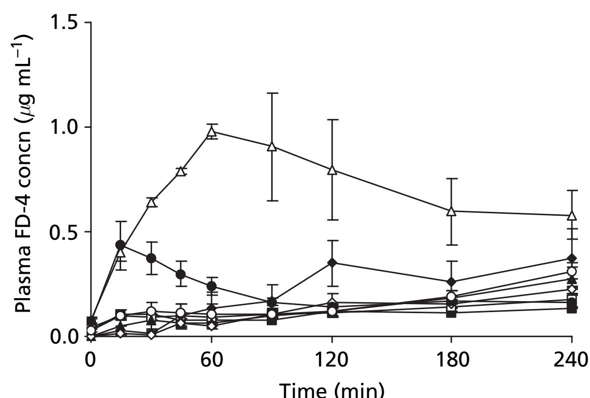


Figure 3 Effects of P2 receptor agonist and purine nucleotides on plasma FD-4 concentrations after FD-4 administration into rat ileum. Symbols: control (■); 1 mM 2-MeSATP (●); 1 mM α,β-meATP (○); 25 mM adenosine (□); 25 mM AMP (▲); 25 mM ADP (△); 25 mM UDP (◇); 25 mM UTP (♦). Each point and the vertical bars represent the mean ± s.e.m. (n = 4).

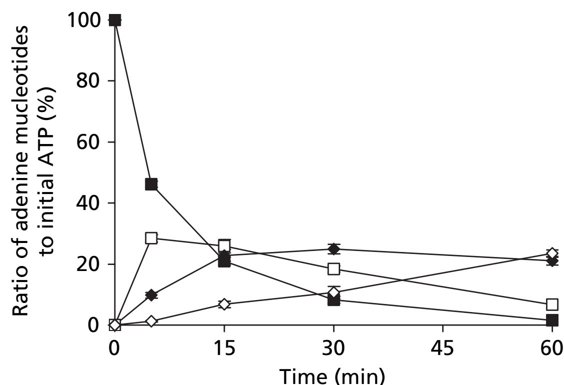


Figure 4 Time course of ATP dephosphorylation in the lumen after ATP (10 mM) administration into rat ileum. Symbols: ATP (■); ADP (□); AMP (♦); adenosine (◇). Each point and the vertical bars represent the mean ± s.e.m. (n = 6).

by 2-MeSATP (1 mM) but not by α,β-meATP (1 mM). Furthermore, the effects of other purine nucleotides on FD-4 absorption were also investigated. Among the purine nucleotides used in this study, only ADP enhanced the absorption of FD-4 (Figure 3). The ADP-induced effect was also inhibited by the presence of suramin (data not shown).

Figure 4 shows the dephosphorylation of ATP after the administration of ATP (10 mM) in the ileum. ATP was rapidly dephosphorylated in the lumen, ultimately to ADP, AMP and adenosine. We further examined the effect of ATP on the transport of FD-4 across the intestine with an in-vitro everted sac method. The transport of FD-4 increased with 1 mM or greater ATP concentration in the mucosal side (Figure 5). The enhanced transport of FD-4 to the serosal side was significantly inhibited by suramin.

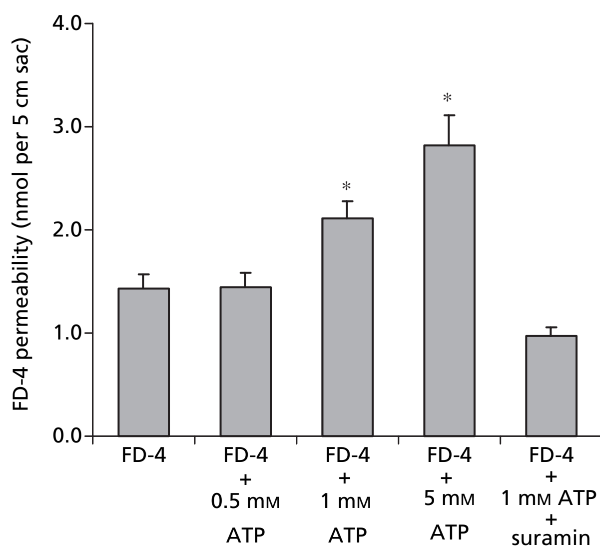
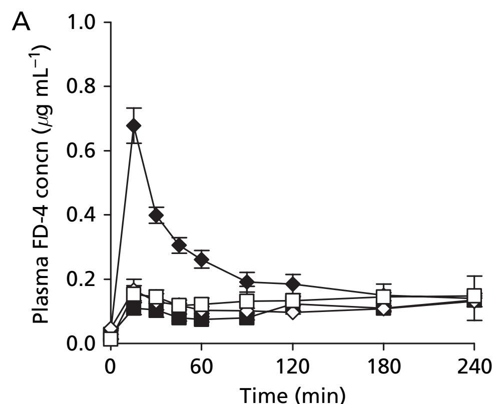


Figure 5 Effects of ATP (0.5–5 mM) and suramin (1 mM) on the permeability of FD-4 (0.25 mM) in rat everted sacs from ileum. Each point and the vertical bars represent the mean ± s.e.m. (n = 4–6, respectively). $P < 0.05$ when compared with FD-4 only.



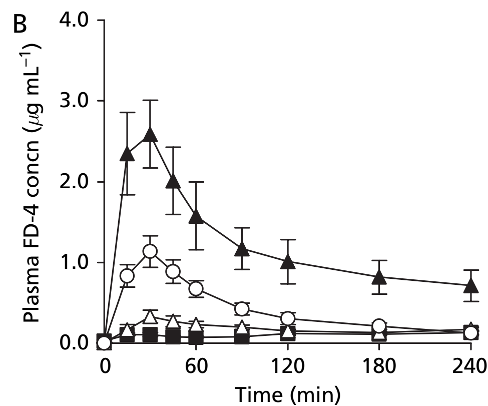


Figure 6 Effects of suramin and PPADS on C12 (A) or DC-Na (B) induced enhancement of FD-4 absorption in rat ileum. Symbols: (A) control (■); 5 mM C12 (♦); 5 mM C12 with 5 mM suramin (◇) or 5 mM PPADS (□). (B) control (■); 5 mM DC-Na (▲); 5 mM DC-Na with 5 mM suramin (△) or 5 mM PPADS (○). Each point and the vertical bars represent the mean ± s.e.m. (n = 4–6, respectively).

Next, we studied the effect of the P2 receptor antagonist on the absorption-enhancing effect of the other absorption enhancers reported previously. C12 and DC-Na were selected as the absorption enhancers because their enhancing effects are well known (Lindmark et al 1995; Sakai et al 1997). These compounds increased the absorption of FD-4 from the ileum. However, the enhancing effects of these compounds were diminished with the co-administration of suramin or PPADS (Figure 6).

Discussion

Extracellular ATP acts on the cell surface P2 receptors of the P2X and P2Y types (Abbracchio & Burnstock 1994; Fredholm et al 1994). P2 receptors are widely expressed in mammalian tissues and regulate a broad range of activities. In a variety of epithelial tissues, including in the intestine, P2 receptors are also found and have been shown to function as important regulators of ion transport (Leipziger 2003). Recently, Tanaka et al (2003, 2005) suggested that the activation of the P2Y receptor enhanced the permeation of marcomolecules through the endothelial cells isolated from the rat caudal artery. However, there are no reports

that have investigated the effects of P2 receptors on the absorption of drugs that are poorly absorbed from the gastrointestinal tract.

In the present study we examined the participation of P2 receptors in the intestinal absorption mechanism and selected FD-4 for use as the poorly absorbed model compound. Indeed, the absorption of FD-4 from rat ileum was found to be poor (Figure 1), but rapidly absorbed when co-administered with ATP (Figure 1). Thus, ATP significantly increased the absorption of FD-4 in a concentration-dependent manner. As one possible explanation for this effect of ATP, mucosal cytotoxicity of the intestine by ATP is postulated. It is well known that extracellular ATP is a broad-spectrum cytotoxic agent that produces an effect via the cell surface P2 receptor. The ligand gated P2 receptor subtype has very high sequence homology with the RP-2 gene, which encodes for apoptosis (Lundy et al 1998). Thus, we evaluated the reversibility of the enhancement effect of ATP by measuring the plasma concentration of FD-4 after pretreatment with ATP. Significant differences were not observed between the control and the pretreatment with ATP groups (data not shown). Additionally, ileum morphological damage was not observed on pathologic examination of the ileum specimen, which was collected after the in-situ loop experiment (data not shown). These results suggest that the enhancement effect of ATP did not result from cytotoxicity.

Suramin and PPADS, both of which are P2 receptor antagonists (Dunn & Blakeley 1988; Lambrecht et al 1992), inhibited the enhancing effect of ATP (Figure 2). These results suggest that the activation of the P2 receptor enhances the absorption of FD-4 from the ileum. P2 receptors are subdivided into two families, P2X and P2Y receptors (Abbracchio & Burnstock 1994; Fredholm et al 1994). P2X receptors are ATP-gate ion channels and P2Y receptors belong to the superfamily of G-protein-coupled receptors (Benham & Tsien 1987; Dubyak 1991). To investigate whether it was the P2X or P2Y receptors that were participating in the absorption enhancing effect of ATP, 2-MeSATP (a P2Y receptor agonist) and α,β-meATP (a P2X receptor agonist) were used. Absorption of FD-4 was enhanced by 2-MeSATP (1 mM), but not by α,β-meATP (1 mM) (Figure 3). These results suggest that the activation of P2Y receptors enhances the absorption of FD-4 from the ileum. To date, at least seven P2Y receptors (P2Y1, P2Y2, P2Y4, P2Y6, P2Y11, P2Y12 and P2Y13) have been identified in various animal species (Ralevic & Burnstock 1998). The present study showed that ATP, 2-MeSATP and ADP enhanced the absorption of FD-4 from the ileum. Other purine nucleotides (AMP, UTP and UDP) and adenosine did not show any enhancing effect. ATP, 2-MeSATP and ADP are all known to be P2Y1 receptor agonists. Our observations suggest that the receptor associated with the enhancing effect of FD-4 absorption may be P2Y1.

The concentration of ATP in cells is about 3 to 10 mM (Schwiebert & Zsembery 2003) under normal physiological conditions. However, in this study, the concentrations of ATP used were higher than those seen under normal conditions. It is well known that ATP is rapidly degraded to ADP, AMP and adenosine by a series of cell surface enzymes (Welford et al 1986; Bailey & Hourani 1990), thus to confirm the degradation of ATP in this study the rate of dephosphorylation of ATP in the rat ileum was measured. ATP was rapidly degraded to ADP, AMP and adenosine (Figure 4). At 30 min, which corresponds to the T_{max} of the plasma FD-4 after ATP co-administration, only 10% of the initial ATP remained in the ileum. From these results it is considered that 5 mM or less of ATP enhances the absorption of FD-4 from the ileum. To further investigate the effect of ATP concentration on the permeation of FD-4, an in-vitro everted sac method was used. The transport of FD-4 increased with 1 mM or greater ATP concentration in the mucosal side (Figure 5). Also, the enhanced transport of FD-4 to the serosal side was significantly inhibited by suramin. Suramin did not affect FD-4 transport. There are many reports that ATP is released from various tissues of the digestive intestine (Matsuo et al 1997; Dezaki et al 2000; Katsuragi et al 2002) but the exact concentration of endogenous ATP released from intestinal cells is not clear. Shinozuka et al (1994) found that alpha-1 adrenoceptor stimulation evoked the release of ATP from blood vessels and the responsible approximation of the amount of ATP per tissue volume that they calculated was 20 μM. ATP is released into the intercellular space from the source cells and this spatial ratio to the whole tissue is very small. The intercellular concentration of ATP is therefore considered to be much higher than 20 μM. Further investigations need to elucidate whether or not endogenous ATP released from intestinal cells affects the transport of a water-soluble high-molecular compound.

Several compounds, including protease inhibitors, surfactants, bile salts, chelating agents and fatty acids, have been developed to increase the intestinal absorption of polar drugs (Lee & Yamamoto 1990; Tomita et al 1996; Sakai et al 1999). It has been reported that the absorption enhancers increase the intracellular calcium levels through interaction with phospholipase C in the membrane, followed by the formation of inositol 1, 4, 5-triphosphate (IP3), and enhance permeability by opening tight junctions, which are opened by activation of the calmodulin-dependent contraction of the actin microfilament (Sakai et al 1998; Ma et al 2000). On the other hand, P2Y receptors generally act via G-protein coupling to activate phospholipase C, followed by the formation of IP3 and an increase in intracellular calcium levels (Benham & Tsien 1987; Dubyak 1991). Tanaka et al (2003) also reported that the P2Y receptor agonist increases the macromolecule permeability of endothelial monolayers with an increase in intracellular calcium. These results suggest the possibility that the absorption enhancer and P2Y receptor agonist enhance the absorption of polar drugs with the same cascade. To elucidate this possibility, we investigated the effect of P2Y receptor antagonist (suramin and PPADS) on the absorption-enhancing effect of C12 and DC-Na. As shown in Figure 6, the absorption-enhancing effects caused by C12 and DC-Na were inhibited with the co-administration of suramin or PPADS. It is considered that the opening tight junctions may be involved in the enhancing effect of the P2Y receptor agonist, although further studies are needed to clarify the detailed mechanism of the P2Y receptor agonist on the absorption enhancing effect of FD-4.

In conclusion, our results demonstrate that extracellular ATP can enhance the absorption of FD-4 from the ileum.

This enhancement effect is caused by the activation of P2Y receptors. Purinergic receptors may therefore participate in the absorption of drugs from the intestine.

References

Abbracchio, M. P., Burnstock, G. (1994) Purinoceptors: are there families of P2X and P2Y purinoceptors? *Pharmacol. Ther*. **64**: 445–475

Anderson, J. M., Van Itallie, C. M. (1995) Tight junctions and the molecular basis for regulation of paracellular permeability. *Am. J. Physiol*. **269**: G467–G475

Bailey, S. J., Hourani, S. M. (1990) A study of the purinoceptors mediating contraction in the rat colon. *Br. J. Pharmacol*. **100**: 753–756

Barthe, L., Bessouet, M., Woodley, J. F., Houin, G. (1998) The improved everted gut sac: a simple method to study intestinal P-glycoprotein. *Int. J. Pharm*. **173**: 255–258

Benham, C. D., Tsien, R. W. (1987) A novel receptor-operated Ca^{2+}-permeable channel activated by ATP in smooth muscle. *Nature* **328**: 275–278

Communi, D., Boeynaems, J. M. (1997) Receptors responsive to extracellular pyrimidine nucleotides. *Trends in Pharmacol. Sci*. **18**: 83–86

Dezaki, K., Tsumura, T., Maeno, E., Okada, Y. (2000) Receptor-mediated facilitation of cell volume regulation by swelling-induced ATP release in human epithelial cells. *Jpn J. Physiol*. **50**: 235–241

Dubyak, G. R. (1991) Signal transduction by P2-purinergic receptors for extracellular ATP. *Am. J. Respir. Cell Mol. Biol*. **4**: 295–300

Dunn, P. M., Blakeley, A. G. (1988) Suramin: a reversible P2-purinoceptor antagonist in the mouse vas deferens. *Br. J. Pharmacol*. **93**: 243–245

Fredholm, B. B., Abbracchio, M. P., Burnstock, G., Daly, J. W., Harden, T. K., Jacobson, K. A., Leff, P., Williams, M. (1994) Nomenclature and classification of purinoceptors. *Pharmacol. Rev*. **46**: 143–156

Hayashi, M., Sakai, T., Hasegawa, Y., Nishikawahara, T., Tomioka, H., Iida, A., Shimizu, N., Tomita, M., Awazu, S. (1999) Physiological mechanism for enhancement of paracellular drug transport. *J. Control. Release* **62**: 141–148

Inoue, C. N., Woo, J. S., Schwiebert, E. M., Morita, T., Hanaoka, K., Guggino, S. E., Guggino, W. B. (1997) Role of purinergic receptors in chloride secretion in Caco-2 cells. *Am. J. Physiol*. **272**: C1862–C1870

Katsuragi, T., Sato, C., Guangyuan, L., Honda, K. (2002) Inositol(1,4,5)trisphosphate signal triggers a receptor-mediated ATP release. *Biochem. Biophys. Res. Commun*. **293**: 686–690

Kimura, T., Murakawa, Y., Ohno, M., Ohtani, S., Higaki, K. (1997) Gastrointestinal absorption of recombinant human insulin-like growth factor-I in rats. *J. Pharmacol. Exp. Ther*. **283**: 611–618

Kimura, Y., Hosoda, Y., Yamaguchi, M., Nagano, H., Shima, M., Adachi, S., Matsuno, R. (2001) Effects of medium-chain fatty acids on intracellular calcium levels and the cytoskeleton in human intestinal (Caco-2) cell monolayers. *Biosci. Biotechnol. Biochem*. **65**: 743–751

Lambrecht, G., Friebe, T., Grimm, U., Windscheif, U., Bungardt, E., Hildebrandt, C., Baumert, H. G., Spatz-Kumbel, G., Mutschler, E. (1992) PPADS, a novel functionally selective antagonist of P2 purinoceptor-mediated responses. *Eur. J. Pharmacol*. **217**: 217–219

Lee, V. H. L., Yamamoto, A. (1990) Penetration and enzymatic barriers to peptide and protein absorption. *Adv. Drug Deliv. Rev*. **4**: 171–207

Leipziger, J. (2003) Control of epithelial tranport via luminal P2 receptors. *Am. J. Physiol*. **284**: F419–F432

Lindmark, T., Nikkila, T., Artursson, P. (1995) Mechanisms of absorption enhancement by medium chain fatty acids in intestinal epithelial Caco-2 cell monolayers. *J. Pharmacol. Exp. Ther*. **275**: 958–964

Lindmark, T., Kimura, Y., Artursson, P. (1998) Absorption enhancement through intracellular regulation of tight junction permeability by medium chain fatty acids in Caco-2 cells. *J. Pharmacol. Exp. Ther*. **284**: 362–369

Lundy, P. M., Sawyer, T. W., Hand, B. T., Frew, R. (1998) Effect of bis-(2-chloroethyl)sulfide on AYP receptor-mediated responses of the rat vas deferens: possible relationship to cytotoxicity. *J. Pharmacol. Exp. Ther*. **285**: 299–306

Ma, T. Y., Tran, D., Hoa, N., Nguyen, D., Merryfield, M., Tarnawski, A. (2000) Mechanism of extracellular calcium regulation of intestinal epithelial tight junction permeability: role of cytoskeletal involvement. *Microsc. Res. Tech*. **51**: 156–168

Madara, J. L. (1989) Loosening tight junctions. Lessons from the intestine. *J. Clin. Invest*. **83**: 1089–1094

Matsuo, K., Katsuragi, T., Fujiki, S., Sato, C., Furukawa, T. (1997) ATP release and contraction mediated by different P2-receptor subtypes in guinea-pig ileal smooth muscle. *Br. J. Pharmacol*. **121**: 1744–1748

Numata, N., Takahashi, K., Mizuno, N., Utoguchi, N., Watanabe, Y., Matsumoto, M., Mayumi, T. (2000) Improvement of intestinal absorption of macromolecules by nitric oxide donor. *J. Pharm. Sci*. **89**: 1296–1304

Ralevic, V., Burnstock, G. (1998) Receptors for purines and pyrimidines. *Pharmacol. Rev*. **50**: 413–492

Sakai, M., Imai, T., Ohtake, H., Azuma, H., Otagiri, M. (1997) Effects of absorption enhancers on the transport of model compounds in Caco-2 cell monolayers: assessment by confocal laser scanning microscopy. *J. Pharm. Sci*. **86**: 779–785

Sakai, M., Imai, T., Ohtake, H., Azuma, H., Otagiri, M. (1998) Effects of absorption enhancers on cytoskeletal actin filaments on Caco-2 cell monolayers. *Life Sci*. **63**: 45–54

Sakai, M., Imai, T., Ohtake, H., Azuma, H., Otagiri, M. (1999) Simultaneous use of sodium deoxycholate and dipotassium glycyrrhizinate enhances the cellular transport of poorly absorbed compounds across Caco-2 cell monolayers. *J. Pharm. Pharmacol*. **51**: 27–33

Schwiebert, E. M., Zsembery, A. (2003) Extracellular ATP as a signaling molecule for epithelial cells. *Biochim. Biophys. Acta* **1615**: 7–32

Shinozuka, K., Hashimoto, M., Masumura, S., Bjur, R. A., Westfall, D. P., Hattori, K. (1994) In vitro studies of release of adenine nucleotides and adenosine from rat vascular endothelium in response to alpha1-adrenoceptor stimulation. *Br. J. Pharmacol*. **113**: 1203–1208

Tanaka, N., Kawasaki, K., Nejime, N., Kubota, Y., Takahashi, K., Hashimoto, M., Kunitomo, M., Shinozuka, K. (2003) P2Y receptor-mediated enhancement of permeation requires Ca^{2+} signaling in vascular endothelial cells. *Clin. Exp. Pharmacol. Physiol*. **30**: 649–652

Tanaka, N., Nejime, N., Kubota, Y., Kagota, S., Yudo, K., Nakamura, K., Kawasaki, K., Kunitomo, M., Takahashi, K., Hashimoto, M., Shinozuka, K. (2005) Myosin light chain kinase and Rho-kinase participate in P2Y receptor-mediated acceleration of permeability through the endothelial cell layer. *J. Pharm. Pharmacol*. **57**: 335–340

Tomita, M., Hayashi, M., Awazu, S. (1996) Absorption-enhancing mechanism of EDTA, caprate and decanoylcarnitine in Caco-2 cells. *J. Pharm. Sci*. **85**: 608–611

Welford, L. A., Cusack, N. J., Hourani, S. M. (1986) ATP analogues and the guinea-pig taenia coli: a comparison of the structure-activity relationships of ectonucleotidases with those of the P2-purinoceptor. *Eur. J. Pharmacol*. **129**: 217–224

Yamamoto, A., Uchiyama, T., Nishikawa, R., Fujita, T. (1996) Effectiveness and toxicity screening of various absorption enhancers in the rat small intestine: effects of absorption enhancers on the intestinal absorption of phenol red and the release of protein and phospholipids from the intestinal membrane. *J. Pharm. Pharmacol*. **48**: 1285–1289