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Effect of adrenergic stimulation on drug absorption *via* passive diffusion in Caco-2 cells

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ABSTRACT

It is well known that the enteric nervous system (ENS) regulates the movement and function of the small intestine, but the effects of ENS on drug absorption from the small intestine still remain to be clarified. Focusing on adrenergic effect, we tried to evaluate how adrenergic stimulation influences the drug absorption *via* passive diffusion using Caco-2 cells as model epithelial cells, a terminal effector of ENS. Adrenaline, an adrenergic agonist, did not affect the transport of small molecules such as antipyrine, phenacetin and mannitol, but decreased the transport of large molecules such as FITC-dextran (FD)-20 and FD-40 without transepithelial electrical resistance (TEER) change. These results suggested that the transport of large molecules *via* paracellular route would be attenuated by adrenergic stimulation. Only clonidine, an α_2-agonist, among selective adrenoceptor agonists decreased FD-40 transport across Caco-2 cell monolayers and the agonist also decreased intracellular cAMP. Furthermore, H-89, a protein kinase A inhibitor, significantly decreased FD-40 transport and dibutyryl cAMP, a cAMP derivative, increased it. These results suggest that the decrease in FD-40 transport would be mainly attributed to the decrease in intracellular cAMP and subsequent decrease in PKA activity *via* α_2-receptor stimulation.

1. Introduction

The small intestine is the organ where most of the nutrients from ingestion are absorbed, and plays an essential role in maintenance of homeostasis. Since the physicochemical properties such as volume, pH, osmolarity and viscosity of luminal contents are always variable, the small intestine has to adapt its own function to such luminal environments to maintain efficient absorption of nutrients. This adaptation is controlled by the enteric nervous system (ENS), which is an intrinsic nervous system extending throughout the length of the gastrointestinal tract.

ENS is recognized as an independent integrative system with structural and functional properties similar to those of the central nervous system, and more than 20 neurotransmitters participate in the neural network (Furness, 2000). They include acetylcholine, monoamines, peptides, amino acids, purines and gasses such as NO (Hansen, 2003). ENS consists of two ganglionated plexuses, the myenteric and submucosal plexuses. Myenteric ganglia are distributed between the outer longitudinal and circular muscle layers, and myenteric neurons mainly regulate contractile activity such as segmentation, mixing movements and peristalsis (Kunze and Furness, 1999; Furness, 2000). On the other hand, submucosal ganglia are embedded in the submucosa, and submucosal neurons are mainly involved with epithelial function and blood flow (Hildebrand and Brown, 1990; Cooke and Reddix, 1994). From the aspect of type of neurons, ENS is composed of sensory, interconnecting and motor neurons. Sensory neurons, expressing sensory receptors on the cell membrane, convert external stimuli to electrical impulse, and release neurotransmitters toward interconnecting neurons. Interconnecting neurons communicate only to other neurons, and provide connection between sensory and motor neurons. Motor neurons project their processes to the smooth muscle or the epithelial cells, and directly regulate their function through release of neurotransmitters.

The effect of ENS on the functions of small intestine has been intensively studied with respect to the regulation of the smooth muscle (Liu and Coupar, 1996; Kunze and Furness, 1999; Furness, 2000; Bayer et al., 2003) or the transport of water and/or electrolytes (Cooke, 1989; Hildebrand and Brown, 1990; Cooke and Reddix, 1994). However, the information about the effect of ENS on drug absorption from small intestine is still very limited (Hayden and Carey, 2000; Neunlist et al., 2003; Hiraoka et al., 2005). The intercellular junctional complexes that are formed between enterocytes, restrict the drug absorption *via* paracellular diffusion (Miyoshi and Takai, 2005). As to the transcellular transport, on the other hand, lipid bilayer and various transporters expressing

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on the membrane participate in the drug absorption *via* passive diffusion and active uptake/efflux, respectively (Ito et al., 2005). These functions of epithelial cells are important determinants for drug absorption from the small intestine, but the mechanisms how the neural stimulation affects the functions remain to be clarified. Therefore, it is important to elucidate the mechanisms for understanding the change in drug absorption under various physiological conditions. Particularly, it has recently been found that the abnormal activities of ENS were associated with some serious intestinal diseases such as inflammatory bowel disease and irritable bowel syndrome (Atkinson et al., 2006; Villanacci et al., 2008). The abnormalities would affect the intestinal permeability and motility, but the details and mechanisms have not been well characterized.

Our previous reports have demonstrated that chronic depletion of serotonin, which is one of the most important neurotransmitters and profoundly related in the regulation of intestinal function (McLean and Coupar, 1998), significantly enhanced the transport activity of P-glycoprotein on the brush border membrane of epithelial cells (Hiraoka et al., 2005). In relation to passive diffusion, we previously reported that the absorption of phenol red, a poorly absorbable compound, is suppressed by the stimulation of adrenergic neuron in the vascular-luminal perfusion study or the *in vitro* transport study using rat small-intestinal sheet (Higaki et al., 2004). Although phenol red was employed as a marker of paracellular transport then, it has been recently suggested that phenol red is secreted by an organic anion efflux system (Itagaki et al., 2005, 2008), which means that the previous results (Higaki et al., 2004) may include the change in the activity of efflux system. Some adrenergic nerves directly penetrate into the intestinal mucosa (Furness and Costa, 1980; Cooke, 1988; Sarna and Otterson, 1989), and noradrenaline is well known to be one of important neurotransmitters in ENS (Cooke, 1994). Furthermore, it has been reported that catecholamines at least partially promote the antisecretory action *via* α_2-receptors located on epithelial cells (Valet et al., 1993). As described above, however, the effect of adrenergic stimulation on the drug transport *via* passive diffusion still remains to be clarified. Therefore, we revaluated the effect of ENS on passive diffusion in the present study with antipyrine and phenacetin as markers of transcellular transport, mannitol and FITC-dextrans as markers of paracellular transport by using Caco-2 cells, a model of intestinal epithelial cells, a terminal effector of ENS.

2. Materials and methods

2.1. Materials

Antipyrine, phenacetin, FITC-dextrans with molecular weights of 4000 (FD-4), 20,000 (FD-20) and 40,000 (FD-40), adrenaline, phenylephrine, clonidine, dobutamine, metaproterenol, dibutyryl cAMP (DBcAMP) and H-89 were purchased from Sigma–Aldrich (St. Louis, MO, USA). [^{3}H]Mannitol was purchased from PerkinElmer Inc. (Waltham, MA, USA). All other reagents were analytical grade commercial products.

2.2. Cell culture

Caco-2 cells, obtained from the cell bank of the Intestine of Physical and Chemical Research (Ibaragi, Japan), were grown in a CO_2 incubator (MCO-175, Sanyo, Tokyo, Japan) maintained at 37 °C, 5% CO_2 and 90% relative humidity, using Dulbecco's Modified Eagle's medium (Sigma–Aldrich) containing 10% fetal bovine serum, 20 μg/mL gentamycin, and 100 U/100 μg/mL penicillin/streptomycin (Sigma–Aldrich). The culture medium was changed every other day. Caco-2 cells were seeded at 1×10^5 on a Transwell (growth area, 1.12 cm^2; Corning Inc., Corning, NY, USA). The cells were grown for 14–20 days for study.

2.3. Transport study

After preincubation with Ringer's solution (pH 7.4) 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 for 25 min, Ringer's solution containing each passive diffusion marker was placed in the apical side (0.5 mL) of Caco-2 cell monolayers on a Transwell. Adrenergic agonists or intracellular signal modulators were added into the basal side (1.5 mL) at the same time. Samples (0.2 mL for antipyrine, phenacetin and mannitol; 0.5 mL for FD-4; 0.75 mL for FD-20 and FD-40) were drawn out of the basal side at 10-min intervals to 90 min. An equal volume of Ringer's solution containing each adjuvant was immediately added to the basal side after each sampling. Transepithelial electrical resistance (TEER) was simultaneously determined using a Millicell-ERS resistance system (Millipore, Billerica, MA, USA).

2.4. Measurement of intracellular cAMP level

After preincubation with Ringer's solution (pH 7.4), adrenaline or clonidine was added into the basal side of Caco-2 cell monolayers on a Transwell. After incubation for 2, 6, 10, 20, 45 or 90 min, the reaction was stopped by adding 35 μL of 1N HCl solution. After 20 min, the suspension was centrifuged for 10 min at $1000 \times g$. The cAMP levels of the supernatant were determined by enzyme immunoassay using cyclic AMP EIA Kit (Cayman Chemical, Ann Arbor, MI, USA). Samples, the cAMP antibody and the tracer (acetylcholinesterase linked to cAMP) were incubated at 4 °C for 18 h in 96-well microplate pre-coated with mouse monoclonal antibody. After washing wells, quantification of the tracer was achieved by measuring its enzyme activity with Ellman's reagent, where the absorbance of final product, 5-thio-2-nitrobenzoic acid, was determined at 410 nm using a microplate reader (Model 680, Bio-Rad Laboratories, Hercules, CA, USA). Protein amount of samples was determined by DC Protein Assay Kit (Bio-Rad Laboratories).

2.5. Analytical method

FITC-dextrans were determined fluorospectrophotometrically at 485 nm for excitation and at 515 nm for emission (F4500 fluorescence spectrophotometer, Hitachi, Tokyo). Radioactivity of [^{3}H]mannitol was determined in a liquid scintillation counter (LS-232, Beckman, Fullerton, CA, USA) after the scintillation medium (Clear-Sol II, Nacalai Tesque, Kyoto, Japan) was added to each sample.

Antipyrine and phenacetin were determined by HPLC, which consists of a model LC-6A HPLC pump (Shimadzu, Kyoto), a model SPD-6A system controller (Shimadzu) set at 254 nm for antipyrine, or set at 243 nm for phenacetin. A Synergi Fusion-RP column (150 mm × 4.6 mm i.d.; particle size, 4 μm; Phenomenex Inc., Torrance, CA, USA) was used at room temperature. The mobile phase for antipyrine was 20 mM phosphate buffer (pH 7.4):methanol (65:35, v/v) delivered at 1.0 mL/min, and that for phenacetin was 0.025% phosphoric acid:methanol (40:60, v/v) delivered at 1.0 mL/min. The concentration range of standard curves was 0.25–50 or 0.1–50 μM for antipyrine or phenacetin, respectively. The squared correlation coefficient for standard curves was over 0.999. The coefficient of variation (CV) ranged from 0.36 to 4.6%.

2.6. Data analysis

The cumulative amount transported to the receptor side was calculated according to the following equation (1):

$$Qt_n = \sum_{n=1}^{n} [VCt_{n-1}] + 1.5Ct_n \quad (1)$$

where Qt_n and Ct_n indicate cumulative amount transported to the receptor side and the concentration in the receptor side at time t_n, respectively. The volume of samples (V) was 0.2 mL for antipyrine, phenacetin and mannitol; 0.5 mL for FD-4; 0.75 mL for FD-20 and FD-40. Apparent permeability coefficient (P_{app}) was calculated by the equation (2):

$$P_{app} = \frac{dQ}{dt}\frac{1}{AC_0} \quad (2)$$

where dQ/dt, A and C_0 reveal the permeation rate, the exposing surface area (1.12 cm^2) and the initial concentration of each passive diffusion marker, respectively.

2.7. Statistical analysis

Results are expressed as the mean ± S.E. of at least four experiments. Analysis of variance (ANOVA) was used to test the statistical significance of differences between groups. Statistical significance in the differences of the means was determined by Dunnett's method.

3. Results and discussion

Several adrenoceptor-subtypes have been identified in intestine from different species, and it has been reported that at least α_1- and α_2-receptors are expressed on the membrane of intestinal epithelial cells (Cooke, 1988; Sarna and Otterson, 1989; Vliet et al., 1990; Valet et al., 1993; Baglole et al., 2006). Furthermore, the antisecretory action of catecholamines is at least partially mediated by the direct stimulation of postsynaptic α_2-receptors located on epithelial cells (Valet et al., 1993; Burks, 1994). In the case of Caco-2 cells, it has been reported that the decrease in short-circuit current, a typical symptom of α_2-receptor stimulation (Hildebrand and Brown, 1992), was caused by clonidine (Hirano et al., 2002). Then, Caco-2 cell monolayers were employed as the terminal effector of ENS to investigate the effect of ENS on the drug transport *via* passive diffusion by using various compounds as passive diffusion markers.

First of all, antipyrine and phenacetin were examined to investigate the effect of adrenaline on the passive diffusion *via* transcellular route. Antipyrine is highly lipid-soluble and well-absorbed from the small intestine. The drug is, therefore, often used as a marker of passive transport through transcellular route (Lacombe et al., 2004; Aoki et al., 2005). Phenacetin was chosen as another marker, because its physicochemical characteristics are similar to those of antipyrine. Although phenacetin is a typical substrate for CYP1A2, the oxidative metabolism is negligible due to the defective oxidative metabolism in Caco-2 cells (Korjamo et al., 2006). The addition of adrenaline at two different concentrations into the basal solution did not change the transport of antipyrine or phenacetin across Caco-2 cell monolayers (Table 1), suggesting that the adrenergic stimulation would not affect the drug transport through transcellular route *via* passive diffusion.

Table 1
Effect of adrenaline on drug transport *via* transcellular route across Caco-2 cell monolayers.

	P_{app}	
	Antipyrine ($\times 10^{-6}$ cm/s)	Phenacetin ($\times 10^{-6}$ cm/s)
Control	37.8 ± 0.9 (100.0 ± 2.3)	43.8 ± 0.6 (100.0 ± 1.4)
+10 μM ADR	38.8 ± 0.5 (102.4 ± 1.2)	40.5 ± 3.1 (92.4 ± 7.1)
+100 μM ADR	38.3 ± 0.7 (101.3 ± 1.9)	43.3 ± 0.4 (98.9 ± 0.8)

Apparent permeability coefficient (P_{app}) was determined by the transport study. The concentration of antipyrine or phenacetin was 50 μM. Adrenaline (ADR) was added to the basal solution. Results are expressed as the mean ± S.E. of at least four experiments.

To estimate the effect of adrenaline on the paracellular transport *via* the passive diffusion, mannitol and FITC-dextrans with various molecular weights were employed as marker compounds. The addition of adrenaline into the basal solution significantly decreased the transport of FD-20 or FD-40 across Caco-2 cell monolayers, while no change in P_{app} values was found for either mannitol or FD-4 (Table 2). Fig. 1(A) and (B) shows the time courses of cumulative amount of transported FD-40 and TEER with or without adrenaline, respectively. Adrenaline immediately attenuated the transport of FD-40 across Caco-2 cell monolayers, and the effect was maintained throughout the experiments (Fig. 1(A)). These results suggested that the transport of large molecules *via* paracellular route would be attenuated by adrenaline added into the basal side of Caco-2 cells.

TEER, well known as an indicator of tight-junctional integrity, was hardly affected by the addition of adrenaline (Fig. 1(B)), suggesting that adrenaline would not drastically change the structure of tight junctions in Caco-2 cells. Although the significant increase in transmucosal electrical resistance by adrenaline was observed in the case of isolated rat small intestine (Higaki et al., 2004), the difference from the present study might be ascribed to the additional effect of submucosal plexus that is kept in the isolated sheet of small intestine (Cooke and Reddix, 1994; Hayden and Carey, 2000), but not in Caco-2 cell monolayers.

It is usually thought that the change in paracellular permeability is correlated with the change in TEER, but it is not the case with several reports (Takahashi et al., 2002; Shao and Kaushal, 2004). Particularly, it would be possible that TEER value does not reflect slight closing of tight junctions as shown in the present study.

As shown in Table 2, adrenaline affected only the transport of larger molecules such as FD-20 and FD-40 but not smaller molecules *via* paracellular route. These results suggest the trans-

Table 2
Effect of adrenaline on drug transport *via* paracellular route across Caco-2 cell monolayers.

	P_{app}			
	Mannitol ($\times 10^{-6}$ cm/s)	FD-4 ($\times 10^{-8}$ cm/s)	FD-20 ($\times 10^{-8}$ cm/s)	FD-40 ($\times 10^{-8}$ cm/s)
Control	1.88 ± 0.11 (100.0 ± 5.8)	5.11 ± 0.36 (100.0 ± 7.1)	1.16 ± 0.02 (100.0 ± 1.6)	0.82 ± 0.09 (100.0 ± 11.0)
+10 μM ADR	1.96 ± 0.14 (104.5 ± 7.7)	4.96 ± 0.57 (97.2 ± 11.1)	1.17 ± 0.02 (101.5 ± 1.6)	0.67 ± 0.10 (82.3 ± 12.4)
+100 μM ADR	1.91 ± 0.22 (102.1 ± 11.9)	5.81 ± 1.14 (113.2 ± 22.4)	0.96 ± 0.04** (82.4 ± 3.2)	0.48 ± 0.09* (58.9 ± 10.9)

Apparent permeability coefficient (P_{app}) was determined by the transport study. The concentration of mannitol, FD-4, FD-20 or FD-40 was 0.1, 1.0, 0.5 or 0.2 mM, respectively. Adrenaline (ADR) was added to the basal solution. Results are expressed as the mean ± S.E. of at least four experiments.

** $p < 0.01$, compared with each control.

* $p < 0.05$, compared with each control.

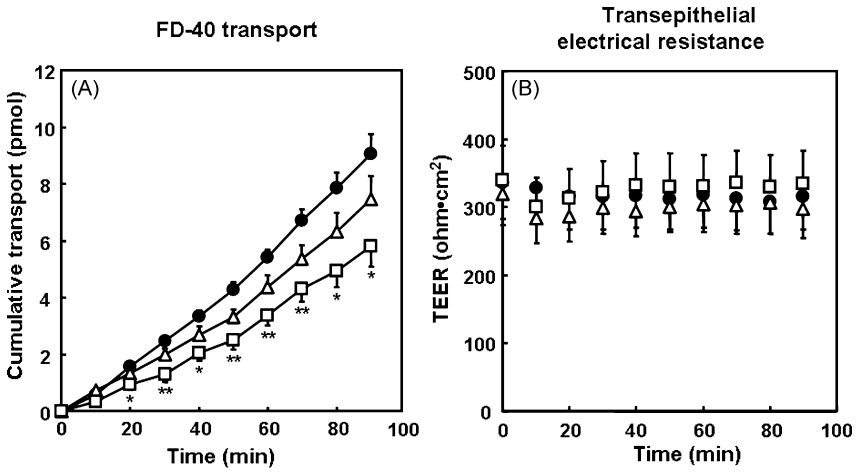

Fig. 1. Effects of adrenaline on FD-40 transport (A) and transepithelial electrical resistance (B) across Caco-2 cell monolayers. Cumulative transport was calculated as described in Section 2. The concentration of FD-40 was 0.2 mM. Adrenaline (ADR) was added to the basal solution. Results are expressed as the mean ± S.E. of at least four experiments. **, $p < 0.01$; *, $p < 0.05$; compared with control. Keys: ●, control; △, +10 μM ADR; □, +100 μM ADR.

port of larger molecules might be more sensitive to the slight modulation of tight junctions, considering that the changes in P_{app} values for FD-20 and FD-40 were much smaller than P_{app} values of mannitol and FD-4 (Table 2). However, further investigation is needed to clarify the mechanisms behind the difference of the regulatory effect by adrenaline between smaller and larger molecules.

Since several adrenoceptor-subtypes are expressed in the intestine (Rang et al., 1999), we examined which subtype of adrenoceptor was involved in the decrease in FD-40 transport by adrenaline (Fig. 2). Phenylephrine (α_1-agonist), clonidine (α_2-agonist), dobutamine (β_1-agonist) and metaproterenol (β_2-agonist) were employed as adrenoceptor-subtype selective agonists. Clonidine significantly decreased FD-40 transport across Caco-2 cell monolayers, while phenylephrine, dobutamine and metaproterenol induced no change in the transport (Fig. 2(A)). Although a higher concentration of clonidine tended to increase TEER, any agonist for each subtype of adrenoceptors did not significantly affect TEER across Caco-2 cell monolayers as did not adrenaline (Fig. 2(B)). These results suggest that the effect of adrenaline would be mainly attributed to the stimulation of α_2-receptor on the basal membranes. Viramontes et al. (2001) reported that the administration of clonidine reduced the colonic sensation without altering gastrointestinal transit in healthy volunteers. Moreover, it has been reported that yohimbine, α_2-antagonist, inhibited the modulation of electrolyte transport by noradrenaline in *in vitro* transport study using rat intestine (Hildebrand and Brown, 1992; Hörger et al., 1998). The antisecretory action of catecholamines is at least partially mediated by the direct stimulation of postsynaptic α_2-receptors located on the epithelial cells (Valet et al., 1993; Burks, 1994). α_2-Receptor in ENS is, therefore, considered to play the major role in the regulation of intestinal function. In the case of Caco-2 cell, a typical electrophysiological symptom of α_2-receptor stimulation was observed (Hirano et al., 2002), and the present study also suggested that α_2-receptor would significantly function even in epithelial cells without neuronal plexuses.

Next, we focused on the intracellular signals on the regulation of functions in Caco-2 cells. The effects of adrenaline on the intracellular cAMP level in Caco-2 cells were evaluated. The AUC of cAMP level$^{0-90min}$ was significantly decreased by the addition of adrenaline (276.4 ± 17.0 pmol min/mg protein, $n = 4$, $p < 0.05$), compared with control (317.1 ± 6.0 pmol min/mg protein, $n = 4$). Clonidine also decreased it (273.7 ± 11.7 pmol min/mg protein, $n = 4$, $p < 0.05$). These results support the observation in Fig. 2, suggesting that the regulation by adrenaline would be mainly attributed to the stimulation of α_2-receptor on the basal membranes. Since the decrease in the intracellular cAMP level leads to the subsequent inhibition of protein kinase A (PKA) (Rang et al., 1999), the effects of PKA inhibition as well as the intracellular cAMP level on FD-40 transport were examined (Fig. 3). H-89, a PKA inhibitor, significantly decreased FD-40 transport across Caco-2 cell monolayers, whereas DBcAMP, a cAMP derivative, significantly

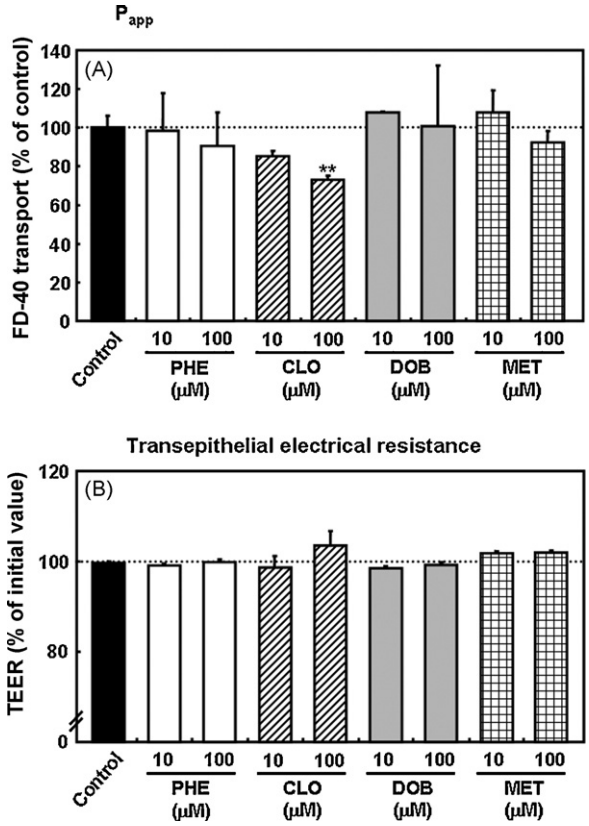

Fig. 2. Effects of adrenoceptor-subtype selective agonists on FD-40 transport across Caco-2 cell monolayers. Apparent permeability coefficient (P_{app}) was determined by the transport study. The concentration of FD-40 was 0.2 mM. Each selective agonist was added to the basal solution. Results are expressed as the mean ± S.E. of at least four experiments. **, $p < 0.01$, compared with control. PHE, phenylephrine; CLO, clonidine; DOB, dobutamine; MET, metaproterenol.

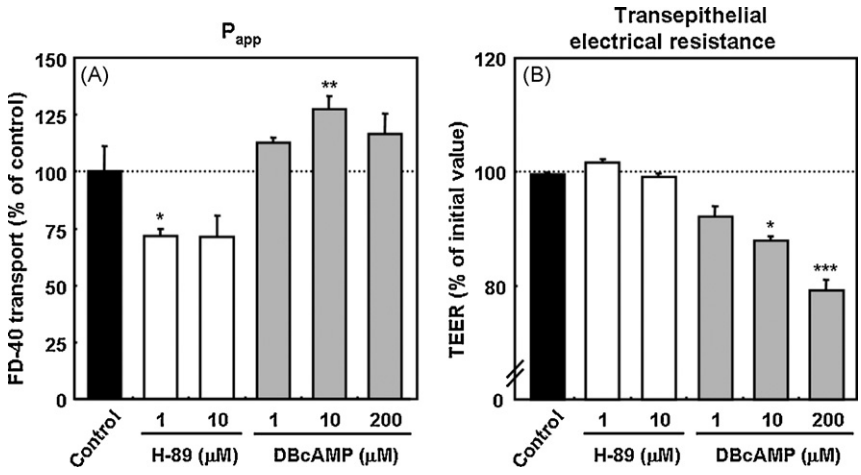

Fig. 3. Effects of intracellular cAMP and PKA on FD-40 transport across Caco-2 cell monolayers. Apparent permeability coefficient (P_{app}) was determined by the transport study. The concentration of FD-40 was 0.2 mM. Each intracellular signal modulator was added to the basal solution. Results are expressed as the mean ± S.E. of at least four experiments. ***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$, compared with control.

increased it (Fig. 3(A)). These results clearly indicate that the intracellular cAMP level, which could be changed by the stimulation of α_2-adrenoceptor, and/or the subsequent change in PKA activity regulates the FD-40 transport. On the other hands, H-89 did not change TEER across Caco-2 cell monolayers, whereas DBcAMP significantly decreased it (Fig. 3(B)). The increase in TEER by DBcAMP might be explained by excessive increase in the intracellular cAMP level.

The details of the mechanisms for the regulation of paracellular transport remain to be clarified, but paracellular diffusion is restricted by the intercellular junctional complexes, mainly tight junctions (González-Mariscal et al., 2003). It has been reported that PKA inhibition by H-89 prevented the loss of membrane-associated staining for tight-junctional proteins induced by low extracellular calcium (Klingler et al., 2000). Furthermore, Pérez et al. (1997) reported that DBcAMP significantly increased the flux of mannitol across rat jejunum. Although either adrenaline or clonidine did not alter TEER and paracellular transport of small molecules across Caco-2 cell monolayers (Table 1 and Figs. 1 and 2), it is possible that they slightly modulated tight-junctional integrity of Caco-2 cells by the decrease in intracellular cAMP level and subsequent decreases in PKA activity.

In addition to tight junctions, adherens junctions also associate the epithelial cells side to side. The disassembly of the adherens junctions does not always cause the decrease in TEER in Caco-2 cells (Barbosa et al., 2003), but it has been reported that cAMP derivatives negatively modulated adherens-junctional integrity of Caco-2 cells, and that H-89 prevented the effect (Boucher et al., 2005). Therefore, the decrease in PKA activity following the decrease in cAMP caused by the stimulation of α_2-receptor on the basal membrane, might induce the tightening of adherens junctions as well.

Some of these junctional proteins are connected with the perijunctional actin (Lapierre, 2000). It has been clearly shown that the elevation of intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) results in the cell contraction through the activation of myosin light chain kinase, thereby reduces the junctional integrity (Ward et al., 2000). It has been reported that intracellular cAMP regulated $[Ca^{2+}]_i$ (Niisato et al., 1997), suggesting that the modulation of $[Ca^{2+}]_i$ might be involved in the adrenergic effect in Caco-2 cells.

In the previous study, we reported that the absorption of phenol red, then used as a marker for passive transport *via* paracellular route, was suppressed by the stimulation of adrenergic neuron in the vascular-luminal perfusion study or the *in vitro* transport study using rat small-intestinal sheet (Higaki et al., 2004). However, the results in Fig. 1 showed that the absorption of the small molecules *via* paracellular route was not affected by adrenaline. It has been recently suggested that phenol red is secreted by an organic anion efflux system (Itagaki et al., 2005, 2008). Although the efflux system has not been clearly identified yet, our previous results might have indicated the modulation of its transport activity and the possible regulation by ENS, the stimulation of adrenergic neurons.

In conclusion, the addition of adrenaline into the basal side of epithelial cells regulates only the absorption of large molecules *via* paracellular route. This regulation would be mainly attributed to the stimulation of α_2-receptor and to the subsequent decrease in the intracellular cAMP level and/or PKA activity. The present results suggest that the intracellular cAMP level and PKA are involved in the regulation of epithelial cells function.

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