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REGULAR ARTICLE

β-Adrenoceptors, but not dopamine receptors, mediate dopamine-induced ion transport in late distal colon of rats

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Abstract Dopamine, an important modulator in the gastrointestinal system, induces concentration-dependent transepithelial ion transport in the distal colon of the rat, as shown by a decrease in the short-circuit current, and acts in a segmentally dependent manner. However, the receptor(s) that mediates dopamine-induced ion transport is unknown. We have investigated the receptor mechanisms underlying dopamine-induced colonic ion transport by means of short-circuit current recording, real-time polymerase chain reaction, and Western blotting analysis, plus gene transfection and enzyme-linked immunosorbance assay. mRNA transcripts of adrenoceptors (α, β) and dopaminergic receptors (D_1 and D_2) were detected in the rat late distal colonic mucosa, with β_2 displaying the highest expression. A similar result was found in human colorectal mucosa (equivalent of late distal colon in rat). Pretreatment with a β_1-adrenoceptor antagonist (CGP-20712A) and a β_2-adrenoceptor antagonist (ICI 118,551) inhibited the dopamine-induced short-circuit current response by 52.59% and 92.51%, respectively. However, neither dopamine D_1 receptor antagonist SCH-23390 nor dopamine D_2 receptor antagonist sulpiride blocked the effect of dopamine. Protein expression of both β_1- and β_2-adrenoceptors was found in the mucosa of rat distal colon and human sigmoid colon and rectum. Dopamine significantly increased intracellular cAMP levels in COS-7 cells transfected with β_1- or β_2-adrenoceptors. Thus, β-adrenoceptors (mainly β_2-adrenoceptors), but not dopamine receptors, mediate dopamine-induced ion transport in the late distal colon of the rat. This extends our knowledge of the late distal colon (rats) or colorectum (human) and provides further experimental evidence that might aid the prevention, diagnosis, and clinical therapy of human colorectal diseases.

Keywords Dopamine · β-Adrenoceptor · Ion transport · Late distal colon · cAMP · Rat (Sprague Dawley) · Human

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Introduction

Catecholamines comprise epinephrine, norepinephrine, and dopamine and are important neurotransmitters in both the peripheral and central nervous systems. Dopamine, as an enteric neurotransmitter, has been identified in mouse, guinea pig (Li et al. 2004), and human (Anlauf et al. 2003). It is not only released from enteric neurons, but can also be synthesized in gastrointestinal epithelial cells and can act as a paracrine modulator of ion transport (Vieira-Coelho and Soares-da-Silva 2001; Anlauf et al. 2003; Tian et al. 2008).

In the mammalian gastrointestinal system, including that of human, dopamine joins in the modulation of manifold functions, including gastrointestinal exocrine secretion (Willems et al. 1985), fluid absorption (Donowitz et al. 1982), intestinal motility (Marzio et al. 1990; Li et al. 2006), blood flow (Kullmann et al. 1983), cytoprotective function (Glavin and Szabo 1990), and immunomodulation (Oberbeck et al. 2006).

Dopamine receptors (D_2, D_4) have been reported to mediate dopamine-induced colonic K^+ secretion across rat distal colon (Al-Jahmany et al. 2004); however, α_2-adrenoceptor has also been reported to be involved in dopamine-induced gastrointestinal water absorption (Donowitz et al. 1983) and ion transport (Vieira-Coelho and Soares-da-Silva 1998; Al-Jahmany et al. 2004). The diverse responses in the different colonic segments can be induced by the same secretory factor (Inagaki et al. 2004; Park et al. 2005), and the different segments within the distal colon also manifest a segmental discrepancy (Yang et al. 2006, 2008; Xue et al. 2007). The colorectum, a narrow part of the colon, is most vulnerable to colorectal inflammation, ulcer, polyposis, and cancer. We have previously reported that dopamine-induced transepithelial ion transport in the late distal colon, the segment close to the anus (corresponding to the colorectum in human) is more prominent compared with other segments of the colon (Zhang et al. 2007). However, the receptor(s) that mediates this response is not known. The purpose of the present study has been to investigate the receptor mechanisms underlying dopamine-induced epithelial ion transport in rat late distal colon.

Materials and methods

Tissue preparation

Adult male Sprague-Dawley rats (weighing 200–300 g; Laboratory Animal Services Center, Capital Medical University, China) had free access to water and standard rat laboratory food until the day of the experiment. Rats were killed by cervical dislocation. The abdomen was opened to expose the intestine, and the distal colonic segment about 7 cm away from the lymph node (typically situated 2 cm from the anus) was quickly removed and immersed in Krebs-Henseleit solution (K-HS). The distal colon was then divided into four segments, termed DC1 (near the lymph node, late distal colon), DC2, DC3, and DC4, respectively. Each segment (1 cm long) was cut longitudinally along the mesenteric border and washed free of luminal contents, and the tissue was pinned mucosal side down in a Sylgard-lined Petri dish containing ice-cold oxygenated solution. The serosa, muscularis, and submucosa were stripped away with fine forceps to obtain the mucosa preparation of the late distal colon.

Specimens of human colon were obtained from patients undergoing surgery for rectal cancer or trauma in the rectum in accordance with the recommendation of the Declaration of Helsinki and with approval of the Ethics Committee at The First Affiliated Hospital of Zhengzhou University, Zhengzhou, China. Specimens were collected in the Department of Anatomical Pathology. The specimens arrived directly from the operating room and generally consisted of a section of the whole wall of the colon from a normal region. They were placed immediately into liquid nitrogen for preservation. When used, the mucosa was quickly stripped from the specimens taken out from liquid nitrogen.

Short-circuit current measurement

The short-circuit current (I_{SC}) was measured in vitro in Ussing chambers. The mucosa preparations were mounted between the two halves of the Ussing chambers (exposed area of 0.50 cm^2), equipped with water-jacketed gas lifts, and were bathed on both sides with 5 ml K-HS, gassed with 95% O_2 and 5% CO_2, pH adjusted to 7.4, and maintained at 37°C by circulating the solution through a reservoir during the experiment. Drugs were added directly to the apical or basolateral side of the epithelial sheets. Responses were continuously recorded by a computer. The transepithelial potential difference for every colonic mucosa was measured by Ag/AgCl reference electrodes (Physiologic Instruments, P2020S) connected to a preamplifer that was, in turn, connected to a voltage-clamp amplifier VCC MC6 (Physiologic Instruments). The change in I_{SC} was calculated on the basis of the value before and after stimulation and was normalized to the current per unit area of epithelium ($\mu A \cdot cm^{-2}$), which allowed the curve area to be calculated ($\mu A \cdot min$). Tissues were incubated for 20–30 min to stabilize the I_{SC} before drugs were added. The baseline value of electrical parameters was determined as the mean over the 3 min immediately before drug administration. All responses were recorded as differences from the baseline (ΔI_{SC}). A positive I_{SC} corresponded to the net eletrogenic secretion of anions (such as Cl^-) or the net electrogenic absorption of cations (such as Na^+).

Solutions and drugs

K-HS contained 117 mmol/l NaCl, 4.7 mmol/l KCl, 24.8 mmol/l $NaHCO_3$, 1.2 mmol/l KH_2PO_4, 1.2 mmol/l $MgCl_2 \cdot 6H_2O$, 2.5 mmol/l $CaCl_2 \cdot 2H_2O$, 11.1 mmol/l glucose. The solution was gassed with a gas mixture of 5% CO_2 and 95% O_2. The pH was adjusted to 7.4 with NaOH or HCl. Drugs that were used in the study included dopamine hydrochloride, indomethacin, dimethyl sulfoxide (DMSO), phentolamine hydrochloride, yohimbine hydrochloride, (±)-propranolol hydrochloride, CGP-20712A, atenolol, ICI

118,551 hydrochloride, R(+)-SCH-23390, sulpiride hydrochloride (Sigma, St. Louis, Mo.), prazosin hydrochloride (Fluka, Buchs, Switzerland), and norepinephrine bitartrate (Jin Yao Amino Acids Pharmaceutical, Tianjin, China). Stock solutions of indomethacin and dopamine were dissolved in DMSO; others were dissolved in aqueous stock solution.

RNA extraction and preparation of cDNA

The distal colonic mucosa was collected in phosphate-buffered saline (PBS; 0.9% NaCl in 0.01 M sodium phosphate buffer, pH 7.4), which had been treated with 0.1% diethyl pyrocarbonate (DEPC-PBS). After the wall of each piece of gut had been opened, the tissue was cleaned with DEPC-PBS and transferred to Trizol (Invitrogen) for extraction of total RNA, which was isolated according to the manufacturer's instructions and stored at -80°C for later use. Samples of cDNA were generated by reverse transcription with 5 μg total RNA, 50 ng random hexamer primers, 10 nM dNTPs, incubated at 65°C for 5 min, and placed on ice for at least 1 min, with the addition of 40 U RNase OUT, 200 U SuperScript III RT, 10 mM dithiothreitole, and 5 mM $MgCl_2$ (Invitrogen), in a 20-μl reaction volume. Following brief centrifugation, the reactions were incubated at 50°C for 50 min and then at 70°C for 15 min. The completed reverse transcription reactions were stored at -20°C and used for the polymerase chain reaction (PCR) without further treatment.

Real time-PCR analysis

Real time-PCR was used to quantify mRNA encoding dopaminergic receptors and adrenoceptors in late distal colon and human rectum. The expression of all the receptors was normalized to that of β-actin, a housekeeping gene, which was not thought to be subject to regulation. The specific primers were as listed in Table 1. Transcripts encoding dopaminergic receptors and adrenoceptors in samples of late distal colon were comparatively quantified by real-time PCR with the Brilliant SYBR Green QPCR Master Mix kit (Stratagene) in a Light Cycler instrument (Stratagene).

Amplifications were performed in a final volume of 20 μl of a commercial reaction mixture (Stratagene). The primers for the amplification of cDNA encoding β-actin and all receptors were used at a final concentration of 0.2 μM. Every 20-μl solution system contained 10 μl Brilliant SYBR Green QPCR Master Mix, 9.05 μl double-distilled water, 0.3 μl cDNA prepared from tissue, 0.05 μl reference dye ROX, and 0.6 μl primer. Within the instrument, the reaction mixture was first incubated at 95°C for 10 min to denature the template DNA. Amplification was then performed for 40 cycles, each involving denaturation at 95°C for 30 s, annealing for 1 min at 60°C, and elongation at 72°C for 30 s. The appearance of double-stranded DNA was quantified by measuring the fluorescence of SYBR Green after each step of annealing. A melting point analysis was finally performed to confirm the sensitivity and specificity of amplification reactions detected with the SYBR Green I

Table 1 Sequences of primers

Primer	Primer sequence	Primer location in the sequence
Rat		
α_{1A}	F:5'-TGG CAG GGT GTT CTG CTG CAA TA-3'	317-336
	R: 5'-GGA CGC TGT GCA GCA TAA GA-3'	355-374
α_{2A}	F: 5'-CTG GCC TCA GCG GAC ATC-3'	223-240
	R: 5'-GTT GGC CAA AGA AAA GGG AAT-3'	259-279
β_1	F: 5'-CAT CAT GGC CTT CGT GTA CCT-3'	714-734
	R: 5'-TGT CGA TCT TCT TGA CCT GTT TCT-3'	755-778
β_2	F: 5'-TTG CCA AGT TCG AGC GAC TAC-3'	372-392
	R: 5'-CAC ACG CCA AGG AGG TTA TGA-3'	411-431
D_{1A}	F: 5'-GGA TGA CAA CTG TGA CAC AAG GTT G-3'	1297-1321
	R: 5'-AAG CTG ATG AGG GAC GAT GAA-3'	1339-1359
D_2	F: 5'-CAC CAC GGC CTA CAT AGC AA-3'	714-734
	R: 5'-GGC GTG CCC ATT CTT CTC T-3'	755-778
Beta-actin	F: 5'-TTC AAC ACC CCA GCC ATG T-3'	419-437
	R: 5'-GTG GTA CGA CCA GAG GCA TAC A-3'	506-527
Human		
β_1	F: 5'-GCG TGT GAT GCA TCT TTA GAT TTT-3'	1807-1830
	R: 5'-CCT AAC CCA CCC ATC TTC CA-3'	1895-1914
β_2	F: 5'-TTG AAG GCC TAT GGG AAT GG-3'	1278-1297
	R: 5'-TCC ACT CTG CTC CCC TGT GT-3'	1315-1334

dye; samples were incubated at 95°C for 1 min, at 55°C for 30 s, and then from 55°C to 95°C with a transition rate of 0.2°C/s. Data were analyzed with computer assistance and MxPro-Mx3000P software.

Western blotting

Tissue was harvested from the mucosa of rat distal colon, washed with PBS, and homogenized in 300 μl cold lysis buffer, pH 7.5, containing Nonidet P-40 (1%), TRIS-HCl (10 mM, pH 8.0), EDTA (1.0 mM), NaCl (150 mM), EGTA (2.0 mM), 10% SDS (0.1%), sodium orthovanadate (1 mM), deoxycholic acid (0.5%), phenylmethanesulfonyl fluoride (1.0 mM), aprotinin (5 μg/ml), and leupeptin (5 μg/ml), all purchased from Sigma. Total tissue homogenates were sonicated to dissolve them completely and then centrifuged at 12,000 rpm for 30 min at 4°C to separate the membrane-containing fraction (pellet) from the cytosol. Proteins (100 μg) were separated by 10% SDS-polyacrylamide gel electrophoresis. The separated proteins were electroblotted onto nitrocellulose membrane (NC membrane, Millipore), and then the membrane was washed for 10 min with TBST (20 mM TRIS-Cl pH 7.5, containing 0.15 M NaCl and 0.05% Tween 20) and immersed in blocking buffer containing 5% nonfat dry milk in TBST for 1 h at room temperature. The blot was washed with TBST and finally incubated overnight at 4°C with polyclonal primary antibodies to β_1 (Affinity BioReagents, USA; diluted 1:500 in 5% nonfat dry milk) or β_2 (Santa Cruz Biotechnology, Santa Cruz, Calif.; diluted 1:500 in 5% nonfat dry milk). After being washed in TBST, the blot was incubated with secondary anti-body to rabbit lgG (Rockland) for 1 h at room temperature. The blot was finally washed with TBST, scanned by infrared rays with the Odyssey Infrared Imager (LI-COR, Nebraska, USA), and analyzed by Odyssey software (version 1.2).

Cell culture and transfection

COS-7 cells were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum, 100 U/ml penicillin, and 100 μg/ml streptomycin (Invitrogen) at 37°C in a humidified atmosphere of 5% CO_2, 95% O_2 in an incubator. Once the cells had reached 80% confluence, they were incubated for 6 h with 4 μg DNA of the β_1-adrenoceptor or 4 μg DNA of the β_2-adrenoceptor (gifts from Junqi He, Beijing, China) and transfection reagent (Hifectin II, Applygen Technologies, China) in different 6-well plates (5×10^5 cells/well) in serum-free media. The serum-free media mixture was subsequently removed, and warm cell culture medium was added. At 48 h after transfection, cells were used for enzyme-linked immunoadsorbent assay (ELISA).

cAMP measurement

cAMP was quantified by ELISA. In brief, after pretreatment with drugs for 10–15 min at 37°C, the media mixture was aspirated; 1 ml 0.1 M HCl was then added to every well. After incubation at room temperature for 20 min, the cells were visually inspected to verify cell lysis. The cell culture mixture was centrifuged at ≥600*g* at room temperature, and the supernatant was used in the assay. cAMP measurement was performed with a cAMP Direct Enzyme Immunoassay kit (Sigma-Aldrich, St. Louis, Mo.) according to the manufacturer's instructions. The optical density (OD) was read at 405 nm on a microplate reader (Bio-Rad).

Statistics

Results are given as arithmetic means±SEM; *n* refers to the number of rats or the number of pairs. Statistical analyses were performed by one-way analysis of variance followed by the Newman-Keuls test or the Student's paired or unpaired *t*-test. Statistics and graphs were generated by using GraphPad Prism, version 4.0 (GraphPad Software, San Diego, Calif., USA). *P*-values less than 0.05 were assumed to denote a significant difference.

Results

Dopamine induced response in I_{SC}

After 20–30 min of stabilization in the Ussing chamber with K-HS, the preparations of the distal colonic mucosa had a mean basal I_{SC} and transepithelial resistance (Rte) of 40.71± 2.12 μA/cm^2 (*n*=61) and 58.55±1.94 Ω*cm^2 (*n*=61), respectively. Endogenous prostaglandins are reported to be released during tissue preparations (Park et al. 2005). Therefore, indomethacin (10 μmol/l), a cyclooxygenase inhibitor, was routinely added to the basolateral side to abolish the effects of remnant endogenous prostaglandins. Basolateral addition of dopamine (100 μmol/l) evoked a decrease in I_{SC} in rat distal colon, and the ΔI_{SC} induced by dopamine was -14.22±2.77 μA•cm^{-2} (*n*=9) in the segment lying 2 cm from anus (late distal colon, DC1), and -3.82±2.00 μA•cm^{-2} (*n*=8) in the segment 7 cm from anus (DC4). Since DC1 manifested the largest response to dopamine (Fig. 1, *P*<0.01), it was therefore chosen to be investigated for the receptor mechanisms of the dopamine-induced response.

Expression of dopaminergic receptors and adrenoceptors mRNA in DC1

Real time-PCR was used to investigate the expression of mRNA encoding dopaminergic receptors (D_{1A}, D_2) or

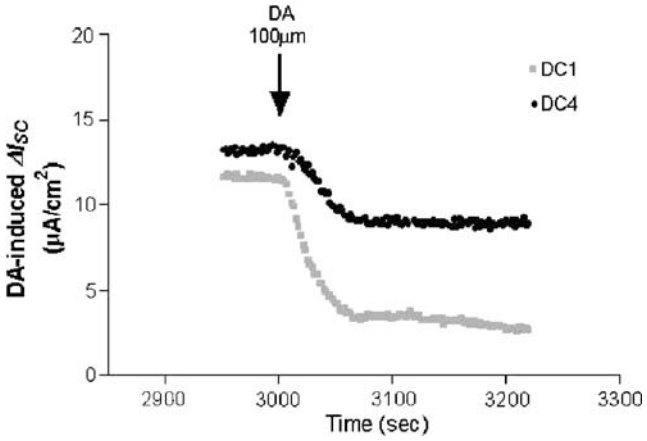

Fig. 1 Dopamine (*DA*) induced a *Isc* response in various segments of rat distal colonic mucosa. The ΔI_{SC} induced by dopamine in the segment 2 cm away from anus (*DC1*) was larger than that recorded in the segment 7 cm away from anus (*DC4*)

adrenoceptors (α_{1A}, α_{2A}, β_1, β_2) in DC1. mRNA transcripts encoding all of the above receptors were found to be expressed in the colonic mucosa of DC1. Quantitative analysis of receptor expression in the mucosa found that the relative abundance of the receptor mRNA was as following: $\beta_2>\beta_1>D_2>\alpha_2>>D_1>\alpha_1$. The mRNA level of the β_2-adrenoceptor was much higher than that of β_1 (Fig. 2a, $P<0.01$); that of the D_1 receptor and α_1-adrenoceptor was extremely low.

mRNA expression of the β-adrenoceptors (β_1, β_2) in the mucosa of human rectum (corresponding to the DC1 segment in the rat distal colon) was also investigated. mRNA transcripts for both β_1 and β_2 receptors were detected in the human rectum, and the mRNA level of β_2 was higher than that of β_1 (Fig. 2b, $P<0.01$), which was similar to the result from DC1 of rat.

Role of dopaminergic receptors in dopamine-induced I_{SC} response

To investigate whether dopaminergic receptor(s) mediate the dopamine-induced decrease in the I_{SC} in DC1, we used antagonists for the D_1-like receptor (SCH-23390) and the D_2-like receptor (sulpiride). Pretreatment with SCH-23390 or sulpiride at concentrations of 0.1, 1, 10, or 100 μmol/l did not inhibit the effect of dopamine (100 μmol/l; Fig. 3), indicating that dopaminergic receptors were not involved in the dopamine-induced decrease in I_{SC}.

Role of adrenergic receptors in dopamine-induced I_{SC} response

In order to investigate whether the dopamine-induced decrease in I_{SC} in DC1 was mediated by adrenergic receptors, norepinephrine (10 μmol/l, added to the serosal side) was used before and after the addition of dopamine. Pretreatment with norepinephrine occluded the dopamine-induced downward deflection of I_{SC}, from -13.17±3.11 μA•cm^{-2} (n=4) to 1.02±1.72 μA•cm^{-2} (n=6; Fig. 4a,b, $P<0.01$). Similarly, pretreatment with dopamine also significantly reduced the norepinephrine-induced I_{SC} response from -36.87±2.97 μA•cm^{-2} (n=9) to -23.77±3.57 μA•cm^{-2} (35.53% inhibition; n=6; Fig. 4a,c, $P<0.001$). Therefore, a common receptor target or a common signaling pathway might exist between dopamine- and norepinephrine-induced colonic ion transportation.

Non-selective and selective adrenoceptor antagonists were used to investigate whether adrenoceptor(s) is involved in dopamine-induced ion transport. Pretreatment with phentolamine (100 μmol/l, serosal side), a non-specific antagonist of α-adrenoceptors, did not significantly affect the dopamine-induced decrease in I_{SC} (Fig. 5a). However, pretreatment with propranolol (5 μmol/l, serosal

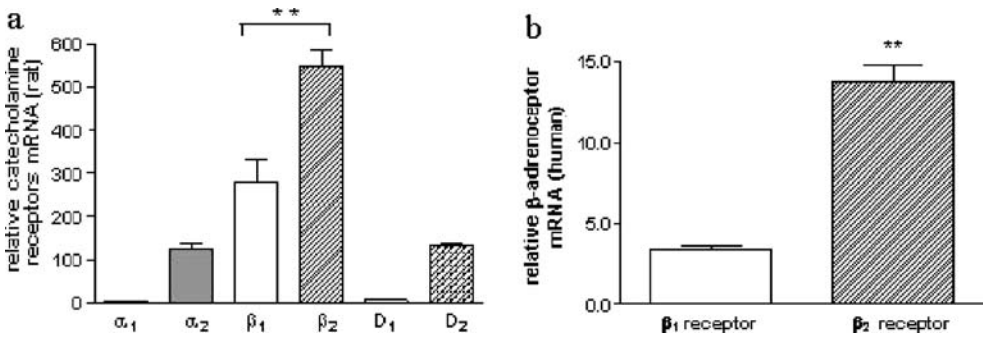

Fig. 2 Expression of adrenoceptor and dopaminergic receptor mRNA in colonic mucosa. **a** mRNA transcripts for both the dopamine receptors (D_1, D_2) and the adrenoceptors (α_1, α_2, β_1, β_2) were detected in rat late distal colonic mucosa. The mRNA level for the β_2-adrenoceptor was much higher than any other receptors. **b** mRNA transcripts for the β_1 and β_2 receptors were also detected in human rectal mucosa; the mRNA level for the β_2 receptor was higher than that of the β_1 receptor. Columns show means±SEM; $^{**}P<0.01$

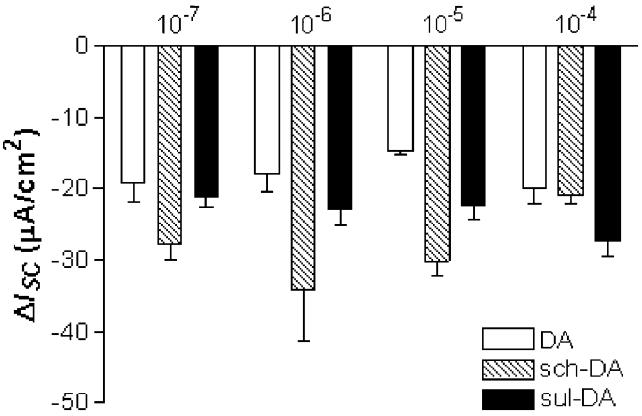

Fig. 3 Effects of dopaminergic receptor antagonists on dopamine-induced decrease in I_{SC}. Pretreatment with SCH-23390 (D_1–like receptor antagonist, n=20) or sulpiride (D_2-like receptor antagonist, n=20) in concentrations of 0.1, 1, 10, or 100 μmol/l did not inhibit the effect of dopamine (100 μmol/l). Columns show the means±SEM (*DA* dopamine, *sch* SCH-23390, *sul* sulpride)

side), a nonselective inhibitor of β-adrenoceptors, reduced dopamine-evoked I_{SC} response by 87.1% (n=6; Fig. 5a, P<0.05), which indicated that the action of dopamine on DC1 might be mediated by β-adrenoceptors. The α_2-adrenoceptor has been reported to be involved in dopamine-induced ion transport in rat jejunum (Vieira-Coelho and Soares-da-Silva 1998) and distal colon (Al-Jahmany et al. 2004). The selective antagonist of the α_2-adrenoceptor, yohimbine, was further tested. Pretreatment of DC1 with yohimbine did not significantly affect the dopamine-induced I_{SC} response (10 μmol/l, serosal side, n=11). However selective inhibitors of the β-adrenoceptors effectively blocked the dopamine-induced I_{SC} deflection. The highly selective β_1-adrenoceptor antagonist CGP-20712A caused concentration-dependent inhibition of the dopamine-induced ΔI_{SC} (Fig. 5b). CGP-20712A at 0.01 μmol/l failed to affect the dopamine-induced response, but it reduced the dopamine-evoked response by 45.35% at 0.1 μmol/l (n=9, P<0.05), by 50.84% at 1 μmol/l (n=7, P<0.01), and by 52.59% at 10 μmol/l (n=9, P<0.01; Fig. 5c). The β_2-adrenoceptor antagonist ICI 118,551 also caused concentration-dependent inhibition of the dopamine-induced I_{SC} response (Fig. 5b). ICI 118,551 at 0.001 μmol/l did not affect the dopamine-induced response, but it suppressed the dopamine-evoked response by 76.29% at 0.01 μmol/l (n=6, P<0.05), by 95% at 0.1 μmol/l (n=12, P<0.001), by 72.79% at 1 μmol/l (n=15, P<0.01), and by 83.15% at 10 μmol/l (n=6, P<0.01; Fig. 5d).

Based on the results obtained with different concentrations of the β-adrenoceptor antagonists, effective antagonist concentrations (CGP-20712A, 0.1 μmol/l; ICI 118,551, 0.01 μmol/l) were used in the following investigations to define the pharmacological receptor profile of dopamine-induced responses. Our previous study showed that dopamine (0.1–1000 μmol/l) evoked a downward deflection in I_{SC} in a concentration-dependent manner with an EC_{50} of 20.06 μmol/l and a maximum response at 100 μmol/l (Zhang et al. 2007). In the present study, we chose the concentrations of dopamine at 1 μmol/l, 10 μmol/l, 50 μmol/l, 100 μmol/l, and 500 μmol/l. The results indicated that both

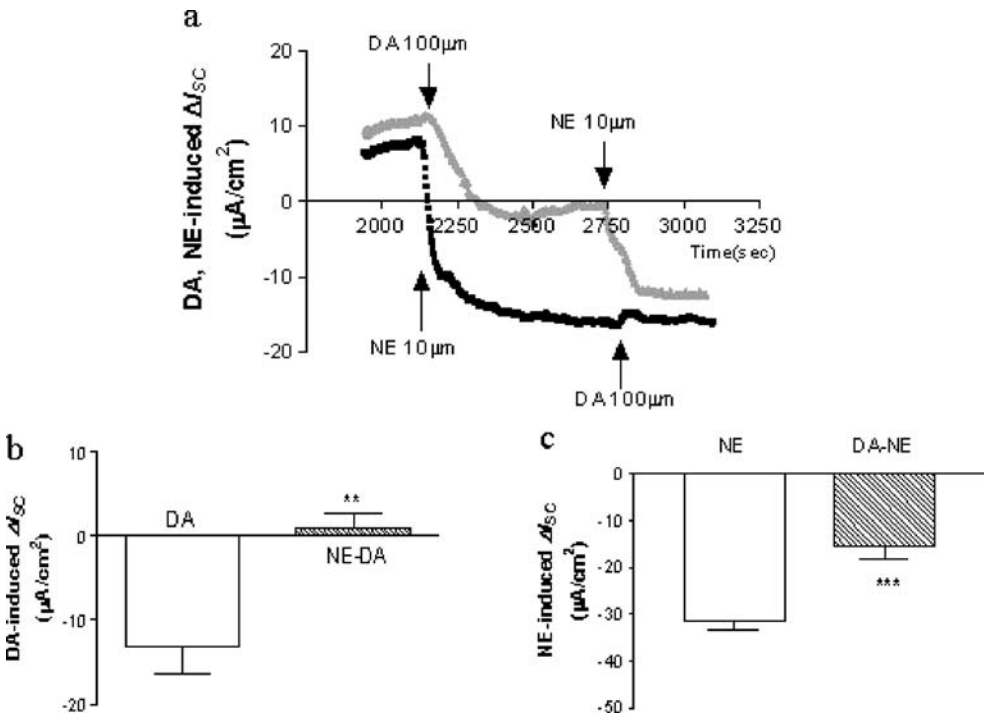

Fig. 4 The interaction of norepinephrine (*NE*) and dopamine (*DA*) on colonic ion transport. **a** In the presence of norepinephrine (10 μmol/l, serosal side), the decrease in I_{SC} induced by dopamine (100 μmol/l, serosal side) disappeared. Similarly, in the presence of dopamine, the ΔI_{SC} evoked by norepinephrine was smaller than the control. **b** The effect of norepinephrine on dopamine-induced ΔI_{SC}. **c** The effect of dopamine on norepinephrine-induced ΔI_{SC}. Columns show the means±SEM; $^{**}P$<0.01; $^{***}P$<0.001

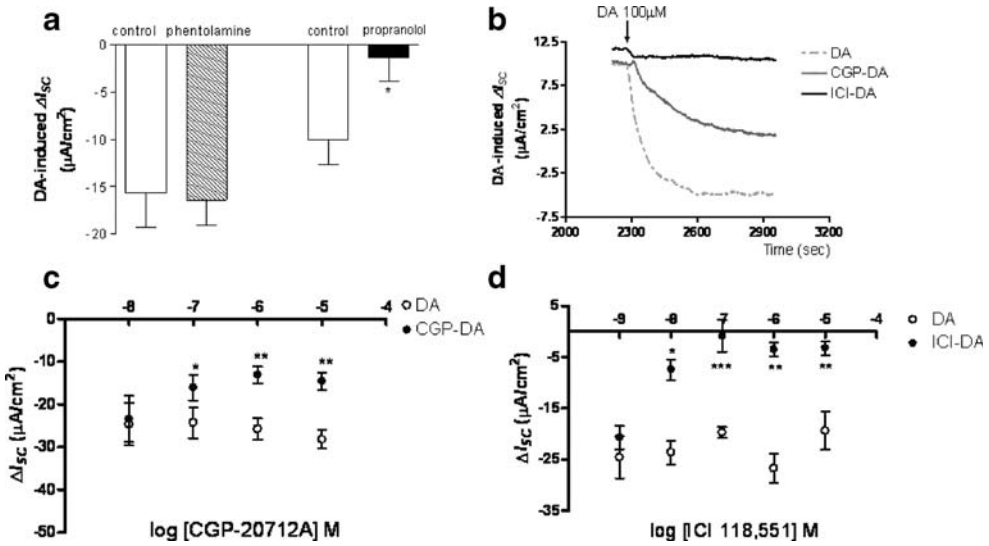

Fig. 5 The effect of selective adrenoceptor antagonists on dopamine-induced I_{SC}. The stripped DC1 segment of the colon from one rat was used as the control, and the corresponding DC1 segment from another rat was used for drug treatment. A paired *t*-test was used for statistical analysis. **a** Effect of nonselective adrenoceptor antagonists on dopamine-induced I_{SC}. Pretreatment with a nonselective α-adrenoceptor blocker, phentolamine (100 μmol/l, serosal side), did not affect the dopamine-induced decrease in I_{SC}. Pretreatment with a β-adrenoceptor antagonist, propranolol (5 μmol/l, serosal side), significantly blocked the effect of dopamine. Columns show the means±SEM. **b** Both the β_1-adrenoceptor antagonist CGP-20712A (*CGP*, 1 μmol/l, serosal side) and the β_2-adrenoceptor antagonist ICI 118,551 (*ICI*, 0.1 μmol/l, serosal side) inhibited the effect of dopamine on I_{SC}. **c** The selective β_1-adrenoceptor antagonist CGP-20712A (0.01–10 μmol/l) concentration-dependently inhibited the effect of dopamine on I_{SC}. **d** The selective β_2-adrenoceptor antagonist ICI 118,551 (0.001–10 μmol/l) concentration-dependently inhibited the effect of dopamine on I_{SC}. In **c**, **d**, each graph shows the concentration-response in the absence (*open bars*) and the presence of antagonists (*closed bars*). *Points* show means, and *vertical lines* indicate SEM, paired *t*-test, $*P<0.05$, $**P<0.01$, $***P<0.001$

CGP-20712A (n=12) and ICI 118,551 (n=13) significantly decreased the dopamine-induced I_{SC} response, and that the extent of the inhibition by ICI 118,551 was stronger than that by CGP-20712A (Fig. 6a,b). The blocking effect of CGP-20712A and ICI 118,551 was surmountable by increasing the concentration of dopamine, suggesting that CGP-20712A and ICI 118,551 were competitive antagonists for the dopamine-induced I_{SC} response.

Since both α- and β-adrenoceptors mediate the ion transport induced by norepinephrine (Schultheiss and Diener 2000), norepinephrine was further investigated as a positive control. The results indicated that, similar to dopamine,

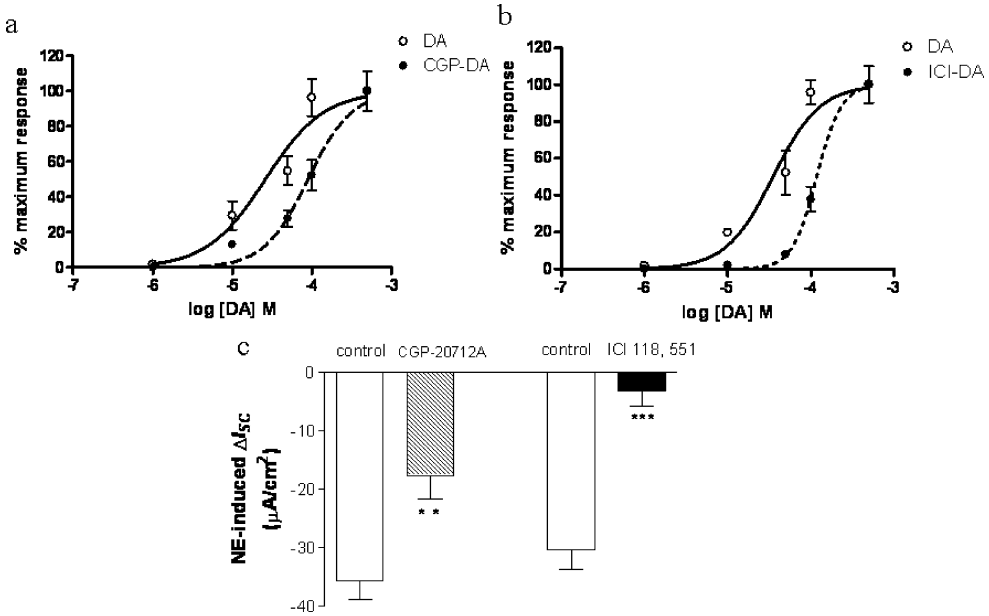

Fig. 6 Concentration-relative response to dopamine on I_{SC} in rat late distal colon. **a**, **b** Response to dopamine in the absence (*open circles*) and presence (*closed circles*) of CGP-20712A (*CGP*; 0.1 μmol/l) and ICI 118,551 (*ICI*; 0.01 μmol/l), respectively. **c** Comparison of norepinephrine (10 μmol/l, serosal side)-induced I_{SC} without (*control*) and with CGP-20712A (1 μmol/l, serosal side) or ICI 118,551 (0.1 μmol/l, serosal side) pretreatment. Columns show the means±SEM, paired *t*-test, $**P<0.01$, $***P<0.001$

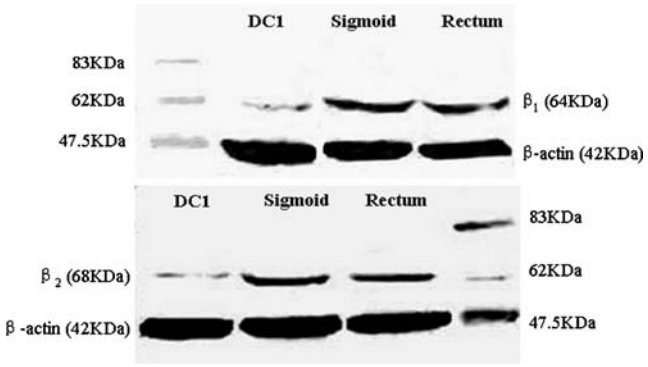

Fig. 7 Protein expression of β_1- and β_2-adrenoceptors as demonstrated by Western blotting. The β_1- and β_2-adrenoceptors are expressed in the mucosa of the rat DC1 segment and in the mucosa of the human sigmoid colon and rectum

norepinephrine-induced colonic I_{SC} decreases in DC1 were significantly inhibited by both CGP-20712A (n=5) and ICI 118,551 (n=6; Fig. 6c), and the β_2-adrenoceptor antagonist ICI 118,551 suppressed the action of norepinephrine by 91.88%.

Expression of β-adrenoceptor protein in DC1 and human colon

Western blotting analysis indicated that both β_1- and β_2-adrenoceptors were demonstrably expressed in the mucosa of rats DC1 and in the sigmoid and rectal mucosa of human (Fig. 7).

Receptor transfection and cAMP measurement

Activation of both the β_1-adrenoceptor and the β_2-adrenoceptor is able to stimulate the generation of intracellular cAMP (Scheid et al. 1979; Kaumann et al. 1989). In order to demonstrate further that dopamine can activate β-adrenoceptors, DNA of the β_1- and β_2-adrenoceptor was respectively transfected into COS-7 cells, a cell line of African green monkey kidney fibroblasts. After transfection for 36–48 h, the protein of the β-adrenoceptor was expressed on the cell membrane (Fig. 8a). Dopamine (100 μmol/l) significantly increased intracellular cAMP levels in both groups without (blank) and with transfection (of β_1-adrenoceptor or β_2-adrenoceptor), and the elevation of intracellular cAMP in transfected groups was much higher than that in the blank groups (P<0.05 for β_1-adrenoceptor, Fig. 8b; P<0.01 for β_2-adrenoceptor, Fig. 8c). Treatment with dopamine increased intracellular cAMP from 1.62±0.12 pmol/ml to 13.81±0.74 pmol/ml (n=3, P<0.001) in COS-7 cells transfected with β_1-adrenoceptor, an 8.5-fold enhancement (Fig. 8b), and from 2.74±0.49 pmol/ml to 27.78±6.44 pmol/ml (n=4, P<0.05) in the β_2-adrenoceptor transfected group, a more than 10-fold increase (Fig. 8c). Norepinephrine might elevate intracellular cAMP levels by activating adrenoceptors and thus was used as a positive control. Addition of norepinephrine (10 μmol/l) increased intracellular cAMP from 1.62±0.12 pmol/ml to 49.36±8.60 pmol/ml (n=3, P<0.01) in β_1-transfected cells, which was much higher than that induced by dopamine (Fig. 8b). In β_2-transfected cells, norepineph-

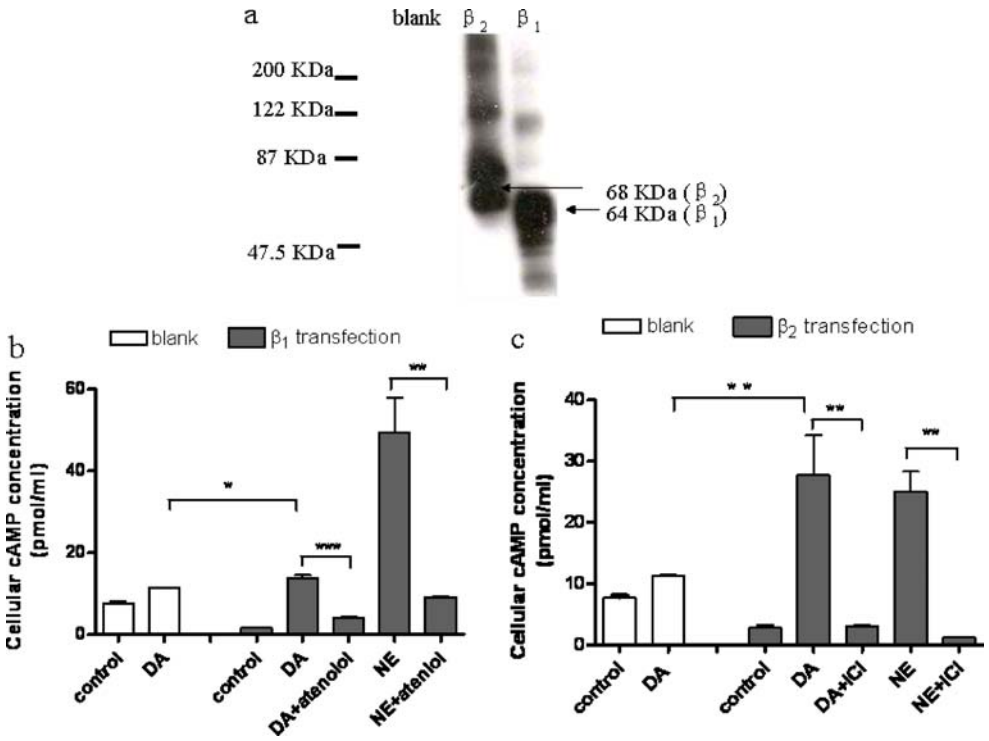

Fig. 8 cAMP measurement in COS-7 cells by ELISA. **a** Western blotting analysis confirmed protein expression of β_1- and β_2-adrenoceptors in COS-7 cells transfected with DNAs for β_1- or β_2-adrenoceptors. **b** In β_1-adrenoceptor-transfected COS-7 cells, both dopamine (100 μmol/l) and norepinephrine (10 μmol/l) increased intracellular cAMP levels. **c** In β_2-adrenoceptor-transfected COS-7 cells, both dopamine (100 μmol/l) and norepinephrine (10 μmol/l) caused an increase in intracellular cAMP levels. Columns show the means±SEM; *P<0.05, **P<0.01, ***P<0.001. Cells without transfection were used as a negative control group. Norepinephrine was used as a positive control. The agonists *atenolol* and ICI 118,551 decreased the responses

rine (10 μmol/l) increased intracellular cAMP from 2.74± 0.49 pmol/ml to 25.04±3.28 pmol/ml (n=3, P<0.01), which was similar to the effect of dopamine (Fig. 8c). These findings suggest that dopamine might be able to bind and activate β-adrenoceptors, and that the binding is much stronger for the β_2-adrenoceptor than for the β_1-adrenoceptor.The two agonists, CGP-20712A and ICI 118,551, once again decreased the responses (Fig. 8b,c).

Discussion

Our previous study has revealed that dopamine is able to evoke transepithelial ion transport in rat late distal colon (Zhang et al. 2007). The dopaminergic receptor has also been found in peripheral organs, including the gastrointestinal tract (Vaughan et al. 2000; Li et al. 2006). However, whether dopaminergic receptor(s) mediates dopamine-induced ion transport in rat late distal colon is not clear. Our present study has demonstrated that β-adrenoceptors, but not dopaminergic receptors, mediate dopamine-induced ion transport in the late distal colon of rats, as evidenced by the following. (1) D_1 and D_2 dopaminergic receptor antagonists, SCH-23390 and sulpiride, fail to inhibit the dopamine-induced I_{SC} response. (2) The nonselective β-adrenoceptor blocker, propranolol, and the selective β-adrenoceptor blockers, CGP-20712A (β_1-adrenoceptor) and ICI 118,551 (β_2-adrenoceptor), inhibit the action of dopamine on I_{SC}, and large amounts of mRNA and protein of the β_1- and β_2-adrenoceptors have been detected in the colonic mucosa of rat late distal colon and in human sigmoid and rectal colon. (3) Pretreatment with norepinephrine totally blocks the subsequent dopamine-evoked decrease in I_{SC}, and pretreatment with dopamine can also weaken the subsequent norepinephrine-induced I_{SC} response, suggesting that dopamine might share a common pathway with norepinephrine. On the other hand, norepinephrine (10 μmol/l) evokes a large decrease in I_{SC} (-36.87±2.97 μA•cm^{-2}), whereas dopamine (100 μmol/l) only evokes a small decrease in I_{SC} (-13.17± 3.11 μA•cm^{-2}). Thus, in this case, norepinephrine works as a full agonist, whereas dopamine might act as a partial agonist on β-adrenoceptors. (4) Dopamine significantly enhances the level of intracellular cAMP in COS-7 cells transfected with either β_1- or β_2-adrenoceptor, similar to the action of norepinephrine.

In the present study, the β_2-adrenoceptor seems to play a more prominent role when compared with that of the β_1-adrenoceptor. The β_2-adrenoceptor antagonist ICI 118,551 at concentration 0.01 μmol/l inhibits the dopamine-induced I_{SC} response, but the β_1 antagonist CGP-20712A is ineffective at the same concentration. When the concentration of CGP-20712A is increased to 0.1 μmol/l, CGP-20712A blocks the dopamine-induced ΔI_{SC} by 45.35%; however, the same concentration of ICI 118,551 reduces dopamine-induced ΔI_{SC} by 95%. The mRNA level of the β_2-adrenoceptor is higher than that of the β_1-adrenoceptor in rat late distal colonic mucosa and human rectal mucosa. In addition, the dopamine-induced increase in intracellular cAMP levels is much higher in β_2-adrenoceptor-transfected COS-7 cells than that in COS-7 cells transfected with β_1-adrenoceptor.

Yu and Ouyang (1997) have found that β-adrenoceptor subtypes are unevenly distributed throughout the gastrointestinal tract, and that β_2-adrenoceptor is the predominant β-adrenoceptor in the mucosa of rat distal ileum, proximal colon, and distal colon. Roberts et al. (1997) have reported that, throughout the colon of human, the mucosa contains moderate levels of β_2-adrenoceptor, low levels of β_1-adrenoceptor, and undetectable amounts of β_3-adrenoceptor mRNA. Our present findings are consistent with these previous reports. In the late distal colonic mucosa, mRNA expression of the β_2-adrenoceptor is the highest among all the catecholaminergic receptors, and activation of the β_2-adrenoceptor demonstrates more prominent action on ion transport than activation of the β_1-adrenoceptor. Stimulation of β-adrenoceptors is well known to cause activation of adenylyl cyclase with a subsequent increase in intracellular cAMP concentration (Scheid et al. 1979; Kaumann et al. 1989), and the β_2-adrenoceptor mediates larger cAMP signals than the β_1-adrenoceptor in human myocardium, because of the stronger coupling to G_s protein compared with the β_1-adrenoceptor (Kaumann and Lemoine 1987; Bristow et al. 1989; Christ et al. 2006). In COS-7 cells transfected with β_1-adrenoceptors, the level of cellular cAMP generated by dopamine is lower than that generated by norepinephrine, but in COS-7 cells transfected with β_2-adrenoceptor, the cellular cAMP increase evoked by dopamine is similar to, or even higher than, that evoked by norepinephrine suggesting that dopamine has a higher affinity for the β_2-adrenoceptor, and that the β_2-adrenoceptor plays a predominant role in dopamine-induced ion transport across the late distal colon in rats.

The five known subtypes of dopamine receptor can be grouped into two families: the D_1-like family (including D_1 and D_5) and the D_2-like family (including D_2, D_3, and D_4; Hartman and Civelli 1997; Tan et al. 2003), which differ in their mode of coupling to adenylate cyclase. Activation of adenylate cyclase via D_1-like receptors leads to an increase in intracellular cAMP. Inhibition of adenylate cyclase via D_2-like receptors causes a reduction of intracellular cAMP (Missale et al. 1998). Dopamine D_{1A} receptor has a transmural distribution in the colon (Vaughan et al. 2000). D_2 receptor has not been found in the epithelium of the mouse distal colon (Li et al. 2006). In our present study, expression of both D_1 and D_2 receptor mRNA transcripts has been detected in the late distal colonic mucosa of the rats, although the expression level is lower than that of the

β-adrenoceptors. However, dopamine receptors seem not contribute to dopamine-induced ion transportation in rat late distal colonic, because antagonists of both D_1-like and D_2-like receptors do not affect the dopamine-induced I_{SC} response. This observation is not consistent with the report of Al-Jahmany et al. (2004), which suggests that D_2 and D_4 receptors are involved in dopamine-induced distal colonic K^+ secretion. The possible reasons for the discrepancy might be attributable to (1) segmental heterogeneity (Yang et al. 2006) as, in our present study, the samples (late distal colon) were collected 2 cm away from the anus, unlike those (distal colon) in the study of Al-Jahmany et al. (2004); (2) the difference in sample preparations as colonic mucosa, but not the mucosa-submucosa, was used in our present study; (3) the drug dosage as the concentration of dopamine in the present study was much lower (100 μmol/l) than that (500 μmol/l) used by Al-Jahmany et al. (2004); (4) the diversity of antagonists as nonselective antagonists of D_1 and D_2 receptor were used in the present study, but the selective antagonist of D_2 or D_4 receptor was used in the study of Al-Jahmany et al. (2004).

Dopamine is well known as being the precursor of norepinephrine and epinephrine in the sympathetic innervation and is able to activate adrenoceptors (Lucchelli et al. 1990; Tsai and Cheng 1992). It has also been reported to stimulate active Na^+ and Cl^- absorption in the rabbit ileum (Donowitz et al. 1982) and to increase ileal and colonic water absorption in the rat by binding to the α_2-adrenoceptor (Donowitz et al. 1983; Vieira-Coelho and Soares-da-Silva 1998). This differs from our data, since the dopamine-induced current response in late distal colon in our present study is not blocked by phentolamine, a nonselective blocker of the α-adrenoceptor, or by yohimbine, a specific antagonist of the α_2-adrenoceptor. The discrepancy might be attributable to the differences of animal species, heterogeneity of tissues, and different sample preparations.

In summary, the present study has demonstrated that dopamine-induced transepithelial ion transport in the late distal colon of rats is mediated by β-adrenoceptors, primarily β_2-adrenoceptors. Dopaminergic receptors seem not to be involved in dopamine-induced transepithelial ion transport in this region of the colon. Our investigation extends our knowledge concerning the morphology and physiology of the rat late distal colon and human colorectum and provides additional experimental evidence as a basis for the prevention, diagnosis, and therapy of human colorectal diseases in clinic.

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