Epidermal growth factor upregulates serotonin transporter and its association with visceral hypersensitivity in irritable bowel syndrome

Xiu-Fang Cui, Wei-Mei Zhou, Yan Yang, Jun Zhou, Xue-Liang Li, Lin Lin, Hong-Jie Zhang

Xiu-Fang Cui, Wei-Mei Zhou, Yan Yang, Jun Zhou, Xue-Liang Li, Lin Lin, Hong-Jie Zhang, Department of Gastroenterology, First Affiliated Hospital of Nanjing Medical University, Nanjing 210029, Jiangsu Province, China

Author contributions: Cui XF, Zhou WM, Yang Y and Zhou J performed the experiments; Cui XF collected and analyzed the data, and wrote the manuscript; Zhang HJ designed the study and revised the manuscript; Li XL and Lin L provided vital guidance to the study.

Supported by National Natural Science Foundation of China, No.81270469; and Key Medical Personnel of Jiangsu Province, No.RC2011063

Correspondence to: Hong-Jie Zhang, MD, PhD, Professor, Department of Gastroenterology, First Affiliated Hospital of Nanjing Medical University, 300 Guangzhou Road, Nanjing 210029, Jiangsu Province, China. hjzhang06@163.com

Telephone: +86-25-83718836 Fax: +86-25-83674636

Received: October 29, 2013 Revised: March 10, 2014

Abstract

AIM: To investigate the role of epidermal growth factor (EGF) in visceral hypersensitivity and its effect on the serotonin transporter (SERT).

METHODS: A rat model for visceral hypersensitivity was established by intra-colonic infusion of 0.5% acetic acid in 10-d-old Sprague-Dawley rats. The visceral sensitivity was assessed by observing the abdominal withdrawal reflex and recording electromyographic activity of the external oblique muscle in response to colorectal distension. An enzyme-linked immunosorbent assay was used to measure the EGF levels in plasma and colonic tissues. SERT mRNA expression was detected by real-time PCR while protein level was determined by Western blot. The correlation between EGF and SERT levels in colon tissues was analyzed by Pearson’s correlation analysis. SERT function was examined by tritiated serotonin (5-HT) uptake experiments. Rat intestinal epithelial cells (IEC-6) were used to examine the EGF regulatory effect on SERT expression and function via the EGF receptor (EGFR).

RESULTS: EGF levels were significantly lower in the rats with visceral hypersensitivity as measured in plasma (2.639 ± 0.107 ng/mL vs 4.066 ± 0.573 ng/mL, P < 0.01) and in colonic tissue (3.244 ± 0.135 ng/100 mg vs 3.582 ± 0.197 ng/100 mg colon tissue, P < 0.01) compared with controls. Moreover, the EGF levels were positively correlated with SERT levels (r = 0.820, P < 0.01). EGF displayed dose- and time-dependent increased SERT gene expressions in IEC-6 cells. An EGFR kinase inhibitor inhibited the effect of EGF on SERT gene upregulation. SERT activity was enhanced following treatment with EGF (592.908 ± 31.515 fmol/min per milligram vs 316.789 ± 85.652 fmol/min per milligram protein, P < 0.05) and blocked by the EGFR kinase inhibitor in IEC-6 cells (590.274 ± 25.954 fmol/min per milligram vs 367.834 ± 120.307 fmol/min per milligram protein, P < 0.05).

CONCLUSION: A decrease in EGF levels may contribute to the formation of visceral hypersensitivity through downregulation of SERT-mediated 5-HT uptake into enterocytes.

© 2014 Baishideng Publishing Group Inc. All rights reserved.

Key words: Epidermal growth factor; Visceral hypersensitivity; Rat models; Serotonin transporter; Rat small intestinal epithelial cells; Intestinal epithelial cells; Irritable bowel syndrome

Core tip: Results of this study show that visceral hypersensitivity results in a decrease in the plasma and colon tissue levels of epidermal growth factor (EGF).
Moreover, the EGF levels were positively correlated with serotonin transporter (SERT) levels. SERT gene expression and protein activity were upregulated in a dose- and time-dependent manner by EGF, and an inhibitor of the EGF receptor kinase blocked SERT gene expression and activity in an intestinal epithelial cell line. The data suggest that decreased EGF levels may contribute to the formation of visceral hypersensitivity through downregulation of SERT activity.

INTRODUCTION

Irritable bowel syndrome (IBS), a common chronic functional gastrointestinal disease, is characterized by abdominal pain and discomfort, and bowel disturbance. The pathogenesis of IBS remains unclear; however, visceral hypersensitivity is the most likely cause for the motor and sensory abnormalities in IBS patients[1,2]. Recent reports indicate abnormalities in serotonergic signaling systems being involved in the development of IBS, particularly those affecting serotonin (5-HT) levels in the gastrointestinal tract[3]. Therefore, it is of interest to investigate the role of this pathway in the pathogenesis of IBS.

High levels of 5-HT have been found in the intestinal mucosal tissue of IBS patients, especially those with constipation[4]. 5-HT is known to facilitate communication between the enteric nervous system and its effector systems (muscles, secretory endothelium, endocrine cells, and vasculature of the gastrointestinal tract). An increase in 5-HT can lead to gastrointestinal motility disorder and visceral hypersensitivity[5]. Accumulating evidence suggests that alterations in serotonergic signaling exist in the gut of IBS patients, including alterations in 5-HT biosynthesis, release, and/or reuptake[6,7].

The serotonin transporter (SERT) is mainly localized to the apical membrane of intestinal epithelial cells. Due to its role in reuptake of 5-HT, SERT plays an important part in terminating transmitter action and maintaining transmitter homeostasis[7,8]. SERT gene expression is downregulated in the colon[9] and rectal tissues[10] of patients with IBS and inflammatory bowel disease. The downregulation may contribute to the pathophysiology of these gastrointestinal disorders; however, the underlying mechanisms are still not fully understood.

Previous studies have demonstrated that epidermal growth factor (EGF) upregulates the reuptake of 5-HT by increasing SERT transcription in human intestinal epithelial cells[11,12]. EGF is a 53-amino acids peptide with a variety of biologic functions. In the gut, EGF plays an important role in intestinal proliferation, differentiation, and maturation[13]. EGF affects various processes by binding to the EGF receptor (EGFR), which is expressed on the basolateral surface of both human and rat intestinal epithelial cells[14] and is associated with certain bowel diseases, such as inflammatory bowel disease[15,16].

Our preliminary findings demonstrated that plasma EGF levels were decreased in IBS patients. To date, the role of EGF in IBS patients remains unknown. Some studies report that SERT-mediated alterations of 5-HT levels in the intestinal space are related to IBS-like syndrome[17,18].

We hypothesized that EGF regulates SERT expression via EGFR and SERT, consequently mediating the 5-HT reuptake, which may be involved in visceral hypersensitivity. In this study, a rodent model of visceral hypersensitivity was established by a two-week colonic infusion of 0.5% acetic acid to produce persistent chronic visceral sensitivity[18-20]. SERT expression was evaluated in this model, and the association of EGF levels with SERT expression was assessed. Lastly, the effect of EGF treatment on SERT expression and its function via EGFR in intestinal epithelial cells were investigated.

MATERIALS AND METHODS

Animals

Sprague-Dawley male rats were purchased from Beijing Vital River Laboratories Animal Technology Co., Ltd. (Beijing, China). Rats were housed with ad libitum food in standard rodent cages at 22 °C in a 12 h light-dark controlled room. All procedures were approved by the Institutional Animal Care and Use Committee of Nanjing Medical University (Nanjing, China), and were in accordance with the guidelines of the International Association for the Study of Pain (IASP; Washington, DC, United States).

Initiation of visceral hypersensitivity

Ten-day-old male rats (n = 10) received an intracolonic infusion of 0.2 mL of 0.5% (v/v) acetic acid in saline, 2 cm from the anus. Control 10-d-old pups (n = 10) were infused with an equal volume of saline alone[19,20]. All of the experiments were conducted on 8-wk-old rats. Two parallel electrodes were implanted in the external oblique muscles of control and colonic-sensitized rats under anesthesia with pentobarbital sodium (50 mg/kg, intraperitoneal). The end of electrode was extended to the back of the necks in both groups for electromyography (EMG) recording. All postoperative rats were housed quietly for seven days in individual cages to recuperate.

Assessment of visceral sensitivity

The visceral sensitivity of the rats was assessed by observing the abdominal withdrawal reflex (AWR) and EMG activity of the external oblique muscle in response to colorectal distension (CRD), as previously described[19,21]. Briefly, the rats were anesthetized with ether, followed by insertion of a flexible balloon, attached to a graded pressure system, into the colon 8 cm from the...
specimens were embedded in paraffin, cut into 4 groups and fixed in 10% (w/v) buffered formalin. Tissue proximal to the anus) were removed from rats of both test/control groups. AWR was scored as follows: 0 = normal behavior with no response, 1 = contraction of abdominal muscles, 2 = lifting of the abdominal wall, and 3 = body arching and lifting of pelvis[20].

**Evaluation of colon inflammation**

After behavior testing, colon tissue specimens (4 cm proximal to the anus) were removed from rats of both groups and fixed in 10% (w/v) buffered formalin. Tissue specimens were embedded in paraffin, cut into 4 µm sections, and stained with hematoxylin and eosin. A pathologist blindly assessed and assigned an inflammatory grade to each section.

**Detection of EGF in plasma and colon tissues**

EGF levels in plasma and colon tissues were quantified using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (R and D Systems Inc., Minneapolis, MN, United States). Following rat anesthetization, 1 mL blood samples were removed from the orbital canthus vein plexus and collected into heparin tubes followed by centrifugation at 1000 × g for 5 min at room temperature. The plasma (supernatant) was then collected and stored at -70 ℃ until analysis. At the same time, colon tissue samples (0.1 g) were homogenized in phosphate-buffered saline with a glass homogenizer on ice. The homogenates were then sonicated with an ultrasonic cell disrupter (New Cheese Biotech Company, NingBo, China) and centrifuged for 5 min at 4 ℃. The supernatants were collected and stored at -70 ℃ until EGF analysis.

**Cell culture**

Rat intestinal epithelial cells (IEC-6) at the 10th culture passage were obtained from the American Type Culture Collection (Manassas, VA, United States) and were cultured as previously described[20]. Briefly, IEC-6 cells were grown in Dulbecco’s Modified Eagle Medium (Gibco of Thermo Fisher Scientific Inc., Waltham, MA, United States), supplemented with 10% FBS (Gibco), 2 mmol/L L-glutamine, 10 mL/L antibiotic solution containing penicillin G (10000 U/mL) and streptomycin (10000 µg/mL; Gibco), and 0.01 mg/mL insulin (Sigma-Aldrich, St. Louis, MO, United States). IEC-6 cells were seeded in polystyrene plastic culture dishes (Corning Inc., Corning, NY, United States), grown for four days (37 ℃ and 5% CO2), and used up to the 25th passage. The medium was changed every 2-3 d. All cell treatments were conducted in serum-free medium following a 1 h period of serum starvation. IEC-6 cell monolayers were treated with 0-160 ng/mL EGF (0203B16; PeproTech, Rocky Hill, NJ, United States) for 0-48 h or with 10 µmol/L EGFR specific kinase inhibitor PD153035 (Sigma-Aldrich) to block EGFR action.

**Western blot detection of SERT**

Intestinal tissue samples were homogenized in potency lisate buffer [25 mmol/L Tris-HCl (pH 7.5); 5 mmol/L EDTA, 5 mmol/L EGTA, 0.5 mmol/L PMSF, 25 µg/mL leupeptin, 10 µg/mL aprotinin, 1 mmol/L sodium vanadate] with a glass homogenizer on ice. Cells were lysed in ice-cold cell lysis buffer [20 mmol/L Tris (pH 7.5), 150 mmol/L NaCl, 1% (w/v) Triton X-100, 0.1% (w/v) sodium pyrophosphate, 1 mmol/L β-glycerophosphate, 1 mmol/L EDTA, 1 mmol/L Na3VO4, 2 µg/mL leupeptin]. Protein concentrations were determined by a BCA protein assay kit (#23250; Thermo Fisher Scientific Inc.). The remaining supernatant (36 µL) was combined (1:1) with 1 × sodium dodecyl sulfate polyacrylamide gel electrophoresis loading buffer, and boiled for 5 min. Approximately 30 µg of protein from each sample was run on a 10% polyacrylamide gel and transferred onto a polyvinylidene difluoride membrane for immunoblotting as previously reported[23,24]. The membranes were blocked with 5% (w/v) non-fat milk, and SERT protein was detected using rabbit anti-SERT polyclonal antibody (AB9726, 1:500; EMD Millipore, Billerica, MA, United States). Mouse anti-GAPDH (Maclesfield, Cheshire, United Kingdom) polyclonal antibody (1:3000) was used as a reference. The membranes were incubated with either horseradish peroxidase-conjugated goat anti-rabbit (BS13278, 1:3000; Bioworld, Louis Park, MN, United States) or goat anti-mouse secondary antibodies (BS12478, 1:3000; Bioworld). All the antibodies were diluted with 5% (w/v) non-fat milk. The SERT/ GAPDH ratio was calculated from the films with the Quantity One Analysis Software (Bio-Rad, Hercules, CA, United States), and the results were expressed in densitometric units.

**Extraction of RNA and real-time PCR detection of SERT mRNA**

The total RNA from control and EGF-treated cells was extracted using TRIzol reagent® (Invitrogen of Thermo Fisher Scientific Inc.). After cDNA was synthesized with a two-step reverse transcription kit (Takara, Dalian, China), real-time PCR was performed on an Applied Biosystems 7500 Real-time PCR System using the SYBR Premix Ex Taq Kit (Applied Biosystems of Thermo Fisher Scientific Inc.) in a 96-well plate. PCR cycle parameters were as follows: initial denaturation at 95 ℃ at 30 s, 40 cycles of 95 ℃ for 5 s and 60 ℃ for 34 s, followed by a dissociation stage for recording the melting curve. The cycle threshold (Ct) values of all genes were obtained, and the relative level of SERT gene was nor-
Cui XF et al. EGF, SERT and visceral hypersensitivity

Figure 1 Effect of neonatal acetic acid treatment on 8-wk-old rat sensitivity to colorectal distension. A: Abdominal withdrawal reflex (AWR) responses to the graded pressures of colorectal distension (CRD) in saline-treated (n = 10) and acetic acid-treated (n = 10) rats. Acetic acid-treated rats show increased AWR scores compared with the saline rats. Values are expressed as mean ± SD. B: Representative electromyogram (EMG) traces recorded in control and acetic acid-treated rats in response to CRD; C: EMG responses to CRD in rats treated with saline and acetic acid at the neonatal stage. Similar to the AWR scores, acetic acid-treated rats exhibited exaggerated EMG activity responses to CRD at different pressures compared with the saline-treated rats. Neonatal rats vs control rats, *P < 0.05, **P ≤ 0.01, error bars represent the mean ± SD.

Statistical analysis

The data were analyzed using SPSS 18.0 software (SPSS Inc., Chicago, IL, United States). Results are expressed as mean ± SD. Two-tailed Student’s t tests were used to analyze differences between two mean values. Two-way repeated measures analysis of variance (ANOVA) was used to evaluate whether the AWR scores and/or EMG area under the curves were altered in the test and/or control groups at different pressures. The remaining data were analyzed using one-way ANOVAs. Values were considered statistically significant if P was < 0.05.

RESULTS

Visceral hypersensitivity development after neonatal colonic sensitization

Neonatal rats (n = 10) treated with 0.5% acetic acid developed visceral hypersensitivity. Compared with the control rats (n = 10), the AWR scores in the acetic acid-treated rats were higher during CRD at all tested distension pressures (Figure 1A). These differences were significant at distension pressures of 40 mmHg (P < 0.01) and 60 mmHg (P < 0.05). Similar to the AWR results, acetic acid-treated rats showed increased EMG responses to CRD at three distension pressures tested (Figure 1B). The EMG area under the curve of the acetic acid-treated rats was significantly higher compared to the controls at 40 mmHg (P < 0.05), 60 mmHg (P < 0.01), and 80 mmHg (P < 0.01) (Figure 1C). No evidence of inflammation or structural abnormalities was found in the control or ac-
tic acid-treated rats, indicating successful establishment of visceral hypersensitivity.

**EGF levels in plasma and colon tissues were lower in rats with chronic visceral hypersensitivity**

ELISA was used to detect any potential differences in EGF levels between the visceral hypersensitive and control group rats. As shown in Figure 2A, the visceral-sensitized rats had significantly lower plasma EGF levels compared with control rats (2.639 ± 0.107 ng/mL vs 4.066 ± 0.573 ng/mL, P < 0.01). The EGF levels in colon tissue from visceral-sensitized rats were also significantly decreased compared with control rats (3.244 ± 0.135 ng/100 mg vs 3.582 ± 0.197 ng/100 mg colon tissue, P < 0.01) (Figure 2B).

**Expression of SERT in colon tissues decreased in rats with chronic visceral hypersensitivity**

Western blot analysis showed a decrease in SERT protein levels in colon tissue of the visceral-sensitized rats (Figure 3). Analysis of the relationship between EGF and SERT levels in colon tissue showed a positive correlation (r = 0.820, P < 0.01).

**Effects of EGF on SERT expression and function in intestinal epithelial cells**

To observe the effect of EGF on SERT levels, IEC-6 cells were treated with various concentrations of EGF for 24 h followed by western blot analysis of SERT protein levels. Our results indicate that EGF upregulates SERT in a dose-dependent manner, with a peak at a dose of 40 ng/mL (Figure 4A). IEC-6 cells were also treated with the optimal dose of EGF (40 ng/mL) for 0, 3, 6, 12, 24, or 48 h. SERT expression was upregulated after a 12 h EGF treatment, with maximal effects after 24-48 h (Figure 4C). The real-time PCR analysis of the EGF effects on SERT gene expression showed that EGF increased SERT mRNA expression in a dose- and time-dependent manner in IEC-6 cells (Figure 4B and D).

A [\(^{3}H\)]-5-HT uptake assay was used to determine whether the high SERT expression, induced by EGF, influenced the reuptake activity of SERT in IEC-6 cells. Uptake of [\(^{3}H\)]-5-HT was significantly higher in cells pre-treated with EGF (40 ng/mL) for 24 h than in the solvent controls (592.908 ± 31.515 fmol/min per milligram vs 316.789 ± 85.652 fmol/min per milligram protein, P < 0.05) (Figure 4E).

**Effects of EGF on SERT gene expression and function via EGFR**

To determine whether the effects of EGF on SERT ex-
Figure 4  Effects of epidermal growth factor on serotonin transporter in rat intestinal epithelial cells. Intestinal epithelial cells (IEC-6) cells were treated with epidermal growth factor (EGF) (0, 20, 40, 60, and 80 ng/mL) for 24 h. A: Western blots were performed to detected serotonin transporter (SERT) protein expressions. GAPDH was used to verify equivalent protein loading (\( ^{a} P < 0.05 \) vs control; \( ^{c} P < 0.05 \) vs 20, 80, and 160 ng/mL); B: SERT gene expression was examined by real-time PCR (\( ^{a} P < 0.05 \) vs control; \( ^{e} P < 0.05 \) vs 20 and 80 ng/mL). To determine the optimal time for EGF treatment, IEC-6 cells were treated with EGF (40 ng/L) for the indicated times (0, 3, 6, 12, 24, and 48 h); C: SERT protein levels were examined by Western blot (\( ^{a} P < 0.05 \) vs control); D: SERT gene expression was examined by real-time PCR (\( ^{a} P < 0.05 \) vs control; \( ^{g} P < 0.05 \) vs 48 h); E: Uptake of [3H]-serotonin in cells pre-treated with 40 ng/ml EGF for 24 h (\( ^{I} P < 0.05 \) vs 24 h). All values are mean ± SD of three independent experiments.
In summary, plasma EGF levels were decreased both in IBS patients and in visceral-sensitized rats, suggesting a potential association of EGF with sensitivity of the gastrointestinal tract. In our preliminary study, the plasma EGF levels in IBS patients were significantly decreased compared with healthy controls (see supplemental Figure 1). Additionally, the levels of EGF in plasma and colon tissues of visceral-sensitized rats were significantly lower than in controls. Furthermore, the Pearson’s correlation analysis showed a positive correlation between EGF and SERT protein levels. These results suggest that EGF may be involved in the development of visceral hypersensitivity via SERT-mediated 5-HT uptake into enterocytes. Consequently, one has to ask how EGF affects SERT gene expression and function. The results of the present study show that EGF upregulates SERT mRNA expression in a dose- and time-dependent manner, suggesting regulation is at the transcriptional level. EGF-stimulated SERT gene expression is known to be dependent on tyrosine kinase activation of EGFR in human choriocarcinoma cells [31]. In this study, inhibition of EGF blocked the effect of EGF on SERT expression and function in IEC-6 cells, thus suggesting that EGF promotes SERT-mediated 5-HT uptake into enterocytes. Clinical studies support the beneficial effects of EGF in decreasing inflammation and diarrhea [32,33]. The beneficial effects of EGF, which may contribute to a change in visceral sensitivity, are worthy of further investigation.

In summary, plasma EGF levels were decreased both in IBS patients and in visceral-sensitized rats, and were correlated with SERT protein expression. Furthermore, treatment of cells with EGF upregulated SERT expression and function via EGFR. These results suggest that
EGF downregulates SERT-mediated 5-HT uptake into enterocytes, potentially contributing to the development of visceral hypersensitivity.

There are some limitations to this study. First, we did not confirm the findings concerning EGFR signaling on SERT expression and change of visceral hypersensitivity in vivo. Future studies are needed to use mice expressing dominant-negative EGFR point mutations to test whether the EGFR signaling pathway is involved in SERT expression and visceral hypersensitivity. Although accumulating evidence indicates that alterations in 5-HT signaling occurs in IBS, the underlying mechanism remains not well understood. Further studies are needed to better understand the pathogenesis of visceral hypersensitivity.

REFERENCES

1. Talley NJ. Spiller R. Irritable bowel syndrome: a little understood organic bowel disease? Lancet 2002; 360: 555-564 [PMID: 12241674 DOI: 10.1016/S0140-6736(02)09712-X]
2. Yan C, Xin-Guang L, Hua-Hong W, Jun-Xia L, Yi-Xuan L. Effect of the 5-HT4 receptor and serotonin transporter on visceral hypersensitivity in rats. Braz J Med Biol Res 2012; 45: 948-954 [PMID: 22832600 DOI: 10.1590/S0100-879X2012000700012]
3. Miwa J, Echizen H, Matsuoka K, Umeda N. Patients with constipation-predominant irritable bowel syndrome (IBS) may have elevated serotonin concentrations in colonic mucosa as compared with diarrhea-predominant patients and subjects with normal bowel habits. Digestion 2001; 63: 188-194 [PMID: 11351146 DOI: 10.1159/000051888]
4. Stasi C, Bellini M, Bazzotti G, Blandizzi C, Milani S. Serotonin receptors and their role in the pathology and therapy of irritable bowel syndrome. Tech Coloproctol 2014; 18: 613-621 [PMID: 24325180 DOI: 10.1016/j.techcol.2013.11.018]
5. Coates MD, Mahoney CR, Linden DR, Sampson JE, Chen J, Blaszyk H, Crowell MD, Sharkey KA, Gershon MD, Mawe GM, Moses PL. Molecular defects in mucosal serotonin content and decreased serotonin reuptake transporter in ulcerative colitis and irritable bowel syndrome. Gastroenterology 2004; 126: 1657-1664 [PMID: 15188158 DOI: 10.1053/j.gastro.2004.03.013]
6. Dunlop SP, Coleman NS, Blackshaw E, Perkins AC, Singh G, Marsden CA, Spiller RC. Abnormalities of 5-hydroxytryptamine metabolism in irritable bowel syndrome. Clin Gastroenterol Hepatol 2005; 3: 349-357 [PMID: 15822040 DOI: 10.1016/S1552-3554(04)00726-8]
7. Latorre E, Mendoza C, Matheus N, Castro M, Grasa L, Mosenero JE, Alcalde AI. IL-10 modulates serotonin transporter activity and molecular expression in intestinal epithelial cells. Cytokine 2013; 61: 778-784 [PMID: 23410504 DOI: 10.1016/j.cyt.2013.01.01]
8. Martel F, Monteiro R, Lemos C. Uptake of serotonin at the apical and basolateral membranes of human intestinal epithelial (Caco-2) cells occurs through the neuronal serotonin transporter (SERT). J Pharmacol Exp Ther 2003; 306: 355-362 [PMID: 12682218 DOI: 10.1124/jpet.103.049668]
9. Faure C, Patey N, Gauthier C, Brooks EM, Mawe GM. Serotonin signaling is altered in irritable bowel syndrome with diarrhea but not in functional dyspepsia in pediatric age patients. Gastroenterology 2010; 139: 240-258 [PMID: 20803355 DOI: 10.1053/j.gastro.2010.03.032]
10. El-Salhy M, Wendelbo I, Gundersen D. Serotonin and serotonin transporter in the rectum of patients with irritable bowel disease. Mol Med Rep 2013; 8: 451-455 [PMID: 23778763 DOI: 10.3892/mmr.2013.1525]
11. Gill RK, Anbazhagan AN, Esmaili A, Kumar A, Nazir S, Malakooti J, Alrefai WA, Sakesen S. Epidermal growth factor upregulates serotonin transporter in human intestinal epithelial cells via transcriptional mechanisms. Am J Physiol Gastrointest Liver Physiol 2011; 300: G627-G636 [PMID: 21275351 DOI: 10.1152/ajpgi.00563.2010]
12. El-Salhy M, Aboul-Nour N, Aguilera J. Serotonin transport is modulated differently by tetanus toxin and growth factors. Neurochem Int 2003; 42: 535-542 [PMID: 12590935 DOI: 10.1016/S0099-1963(02)01887-9]
13. Dvorak B. Milk epidermal growth factor and gut protection. J Pediatr 2010; 156: 501-505 [PMID: 20105660 DOI: 10.1016/j.jpeds.2009.11.018]
14. Niederlechner S, Baird C, Petrie B, Wischmeyer E, Wischmeyer PE. Epidermal growth factor receptor expression and signaling are essential in glutamine’s cytoprotective mechanism in heat-stressed intestinal epithelial-6 cells. Am J Physiol Gastrointest Liver Physiol 2013; 304: G543-G552 [PMID: 23275616 DOI: 10.1152/ajpgi.00418]
15. Alexander RJ, Panja A, Kaplan-Liss E, Mayer L, Raicht RF. Expression of growth factor receptor-encoded mRNA by colonic epithelial cells is altered in inflammatory bowel disease. Dig Dis Sci 1995; 40: 485-494 [PMID: 7895532]
16. Dube PE, Yan F, Puniti S, Girish N, McElroy SJ, Washington MK, Polk DB. Epidermal growth factor receptor inhibits colitis-associated cancer in mice. J Clin Invest 2012; 122: 360-370. doi: 10.1172/JCI62278
Cui XF et al. EGF, SERT and visceral hypersensitivity

2780-2792 [PMID: 22772467]

17 Kerckhoffs AP, ter Linde JJ, Akkermans LM, Samsom M. SERT and TPH-1 mRNA expression are reduced in irritable bowel syndrome patients regardless of visceral sensitivity state in large intestine. Am J Physiol Gastrointest Liver Physiol 2012; 302: G1053-G1060 [PMID: 22323131 DOI: 10.1152/ajpgi.00153.2011]

18 Kerckhoffs AP, Ter Linde JJ, Akkermans LM, Samsom M. Trypsinogen, serotonin transporter transcript levels and serotonin content are increased in small intestine of irritable bowel syndrome patients. Neurogastroenterol Motil 2008; 20: 900-907 [PMID: 18363639 DOI: 10.1111/j.1365-2982.2008.01100.x]

19 Xu GY, Shenoy M, Winston JH, Mittal S, Pasricha PJ. P2X receptor-mediated visceral hyperalgesia in a rat model of chronic visceral hypersensitivity. Gut 2008; 57: 1230-1237 [PMID: 18270243 DOI: 10.1136/gut.2007.134221]

20 Winston J, Shenoy M, Medley D, Naniwadekar A, Pasricha PJ. The vanilloid receptor initiates and maintains colonic hypersensitivity induced by neonatal colon irritation in rats. Gastroenterology 2007; 132: 615-627 [PMID: 17258716 DOI: 10.1053/j.gastro.2006.11.014]

21 Al-Chaer ED, Kawasaki M, Pasricha PJ. A new model of chronic visceral hypersensitivity in adult rats induced by colon irritation during postnatal development. Gastroenterology 2000; 119: 1276-1285 [PMID: 11054385 DOI: 10.1053/gast.2000.19576]

22 Guler S, Collins JF. Silencing the Menkes copper-transporting ATPase (Atp7a) gene in rat intestinal epithelial (IEC-6) cells increases iron flux via transcriptional induction of ferroportin 1 (Fpn1). J Nutr 2014; 144: 12-19 [PMID: 24174620 DOI: 10.3945/jn.113.183160]

23 Gendron FP, Mongrain S, Laprise P, McMahon S, Dubois CM, Blais M, Asselin C, Rivard N. The CDC2 transcription factor regulates furin expression during intestinal epithelial cell differentiation. Am J Physiol Gastrointest Liver Physiol 2006; 290: G310-G318 [PMID: 16299403 DOI: 10.1152/ajpgi.00217.2005]

24 Gordillo-Bastidas D, Oceguera-Contreras E, Salazar-Montes A, González-Cuevas J, Hernández-Ortega LD, Armendáriz-Borunda J. Nrf2 and Snail-1 in the prevention of experimental liver fibrosis by caffeine. World J Gastroenterol 2013; 19: 9200-9033 [PMID: 24379627 DOI: 10.3748/wjg.v19.i47.9020]

25 Koldzic-Zivanovic N, Seitz PK, Cunningham KA, Thomas ML, Hughes TK. Serotonin regulation of serotonin uptake in RNA46A cells. Cell Mot Biol Neurobiol 2006; 26: 979-987 [PMID: 16856637 DOI: 10.1017/s10571-006-9007-x]

26 Rao RK. Biologically active peptides in the gastrointestinal lumen. Life Sci 1991; 48: 1685-1704 [PMID: 2020253]

27 Rao R, Porreca F. Epidermal growth factor protects mouse ileal mucosa from Triton X-100-induced injury. Eur J Pharmacol 1996; 303: 209-212 [PMID: 8813570 DOI: 10.1016/0014-2999 (96)00186-0]

28 Rao RK, Thomas DW, Pepperd S, Porreca F. Salivary epidermal growth factor plays a role in protection of ileal mucosal integrity. Dig Dis Sci 1997; 42: 2175-2181 [PMID: 9365155 DOI: 10.1023/A:1011885552989]

29 Tepperman BL, Vozzolo BL, Soper BD. Effect of maternal si-ladenedectomy on ontogenic response of rat gastric mucosa to luminal H+. Am J Physiol 1993; 265: G354-G360 [PMID: 836917]

30 Kubota N, Kiuchi Y, Nemoto M, Oyamada H, Ohno M, Funahashi H, Shioda S, Oguchi K. Regulation of serotonin transporter gene expression in human glial cells by growth factors. Eur J Pharmacol 2001; 417: 69-76 [PMID: 11301061 DOI: 10.1016/S0014-2999(01)0006-2]

31 Kekuda R, Torres-Zamorano V, Leibach FH, Ganapathy V. Human serotonin transporter: regulation by the neuroprotective agent aminotriacarboxylic acid and by epidermal growth factor. J Neurochem 1997; 68: 1443-1450 [PMID: 9084414 DOI: 10.1046/j.1471-4159.1997.6804143.x]

32 McCole DF, Rogler G, Varki N, Barrett KE. Epidermal growth factor partially restores colonic ion transport responses in mouse models of chronic colitis. Gastroenterology 2005; 129: 591-608 [PMID: 16083715 DOI: 10.1016/j.gastro.2005.06.004]

33 Rieger M, Sedivy R, Sogukoglu T, Cosentini E, Bischof G, Teleky B, Feil W, Schiessl R, Hamilton G, Wenzl E. Effect of growth factors on epithelial restitution of human colonic mucosa in vitro. Stand J Gastroenterol 1997; 32: 925-932 [PMID: 9299673]

P- Reviewer: Troncoso MF, Yang P S- Editor: Ma YJ L- Editor: O’Neill M E- Editor: Wang CH
