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Original Article

Activation of 5-HT and NR2B contributes to visceral hypersensitivity in irritable bowel syndrome in rats

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Abstract: The roles of 5-hydroxytryptamine (5-HT) and spinal N-methyl-D-aspartic acid receptor 2B (NR2B) in visceral hypersensitivity were investigated. A rat model with irritable bowel syndrome (IBS) was established by intracolonic injections of acetic acid onpost-natal days 8-21. Rats were randomly divided into five groups: normal intact (control) group, IBS model group, Ro25-6981-treated IBS rats (Ro25-6981, a NR2B antagonist) group, amitriptyline-treated IBS rats (amitriptyline, a 5-HT antagonist) and Ro25-6981 plus amitriptyline-treated IBS rats (Ro25-6981+amitriptyline) group. The expressions of 5-HT, NR2B, 5-HT2AR, 5-HT7R, SERT, TNF-α and IL-1β in colon, dorsal root ganglion (DRG) and hypothalamus, respectively, were measured by Immunohistochemical staining, Real-Time Reverse Transcription-PCR and Western blotting. Our results showed increased DRG and hypothalamus expression of 5-HT, NR2B, 5-HT2AR, 5-HT7R in IBS model group and decreased expression of those in Ro25-6981 and amitriptyline alone or both treatment groups. Moreover, SERT expression was decreased in colorectal, DRG and hypothalamus of ISB model rats, but increased by Ro25-6981 and amitriptyline alone or both treatments. Ro25-6981 and amitriptyline treatment also decreased colorectal expression of TNF-α and IL-1β induced by IBS model. In conclusion, activation of 5-HT and NR2B may play a crucial role in visceral hypersensitivity in irritable bowel syndrome in rats.

Keywords: Irritable bowel syndrome, visceral hypersensitivity, 5-HT, NR2B, 5-HT2AR, 5-HT7R

Introduction

Irritable bowel syndrome (IBS) is one of the most common chronic disorders referred to gastroenterologists, which characterized by abnormal discomfort or pain, a change in bowel habit and a high prevalence of increased anxiety [1, 2]. It is likely that various factors contribute to the causation of IBS, including inflammatory reactions, gastrointestinal dysmotility, hypersensitivity and genetic variations [3, 4], but no final mechanisms have yet been agreed upon. Visceral hypersensitivity plays a role in some dyspeptic patients and this abnormality is also a potential target for treatment. Visceral hypersensitivity has been described as a hallmark of IBS [5]. Over one-third of IBS subjects demonstrate some degree of hypersensitivity, with either lower pain thresholds and/or higher intensity of sensations [6, 7]. Epidemiological surveys have demonstrated different estimation of prevalence of visceral hypersensitivity in patients with IBS which varied from 33% to 90% [8], and the severity of IBS complications in patients with hypersensitivity IBS is dramatically higher than other IBS patients [9].

The pathophysiology of IBS is incompletely understood; altered brain-gut interactions are thought to play an important role in the cardinal symptoms, particularly abdominal pain [10]. Particularly, clinical evidence suggest that in complex and multifactorial diseases such as IBS, psychological disorders represent significant factors in the pathogenesis and course of the syndrome [11]. The brain-gut axis is complex and involves multiple systems including hypothalamus, and stimulated by various central nervous system (CNS)-and gut-directed stressors. Once a stimuli is applied, afferent

information travels throughout nerve. First relay occurs at dorsal root ganglia (DRG), through which they reach the intestinal wall plexi in the colon and rectum, and through a complex distribution to the anal sphincter [12, 13].

Several genetic disturbances related to IBS have been identified. The neurotransmitter 5-HT is widely distributed throughout the brain and gut axis and has been linked to the regulation or modulation of several symptoms in several subtypes of IBS [14]. 5-HT uptake mechanism possesses a key role in the production of effective treatment in visceral hypersensitivity associated disorders [15]. N-methyl-D-aspartate (NMDA) receptors, including NR1, NR2 (A-D) and/or NR3 (A, B), are involved in the formation and development of chronic visceral hyperalgesia. The expression of NR2B was significantly increased in the spinal dorsal horn in a chronic visceral hyperalgesia rat model versus the control group [16], but the potential mechanism of its action remains to be clarified. Besides, intrathecal injection of the selective NR2B receptor antagonist, Ro25-6981, can dose-dependently inhibit neuropathic pain without causing motor dysfunction [17].

In the present study, an IBS-model rat was established by acetic acid stimulation on postnatal days 8-21. The expression of 5-HT, 5-HT transporter protein (SERT), NR2B, 5-HT2AR and 5-HT7R was analyzed by Immunohistochemistry, Real-Time Reverse Transcription-PCR and Western blot. Effects of the selective antagonist of NR2B and 5-HT on the expression of 5-HT, SERT, NR2B, 5-HT2AR and 5-HT7R were also evaluated.

Materials and methods

Animals

Male Sprague-Dawley neonatal rats (360-500 g, age <8 days) were purchased from the Tongde Hospital of Zhejiang Province. The animals were housed with micro-isolator cages equipped with filter hoods, under controlled temperature (20°C), with a light/dark cycle of 12 h light and 12 h dark, and free access to food and water. The experiments were approved by the Animal Care and Use Committee of Tongde Hospital of Zhejiang Province.

Animal model of visceral hyperalgesia

In order to investigate mechanisms of 5-HT and NR2B in IBS progression, the visceral hyperalgesia model was induced based on an IBS model reported by Al-Chaer et al [18]. Neonatal rats in group 1 (n=5) received intracolonic injections of acetic acid (1 mL, 0.2-0.5%) daily between the ages of 8 and 21 days. Acetic acid was injected into the colon via PE90 tubing inserted to 2 cm from the anus. Rats in group 2 (control, n=5) were handled similarly to those in IBS model groups except that same volumes of 0.9% saline was injected into the colon. Rats in this group were gently held and touched on the perineal area daily between the ages of 8 and 21 days. Group 2 served as a control for colon irritation and is referred to as the control group. Rats in group 3 (n=5) intragastric administration of amitriptyline (10 mg/kg) a daily basis after IBS model was established for 35 days. Rats in group 4 (n=5) intraperitoneal injection of Ro25-6981 (20 μl) a daily basis after IBS model was established for 35 days. Rats in group 5 (n=5) received both amitriptyline and Ro25-6981 administration similarly to those in groups 3 and 4. After amitriptyline and/or Ro25-6981 were treated for 4 weeks, the rats were deeply anesthetized with an intraperitoneal injection of 10% chloral hydrate (0.3 mL/100 g).

Immunohistochemical staining of 5-HT, NR2B, 5-HT2AR, 5-HT7R, TNF-α and IL-1β

At the end of the treatment, 1.5 cm×1.5 cm×0.3 cm of the colon, DRG and hypothalamus was removed. A cross section of the colon, DRG and hypothalamus tissues was fixed in 10% formalin, dehydrated in graded alcohols and xylene, embedded in paraffin, and cut serially into 4-7 μm for immunohistochemical staining. After dewaxing and rehydration, the sections were antigen-retrieved in 10 mm citrate buffer for 5 min at 100°C. Endogenous peroxidase activity and non-specific antigens were blocked with 3% hydrogen peroxide and serum. The slices were subsequently incubated with 5-HT (1:400, ab66047, Abcam), NR2B (1:1000, ab93610, Abcam), 5-HT2AR (1:500, Sc-15073, Santa cruz), 5-HT7R (1:500, Sc-28963, Santa cruz), TNF-α (1:100, ab6671, Abcam) and IL-1β (1:100, ab9722, Abcam) antibody overnight at 4°C. Slides were then incubated with goat anti-rab-

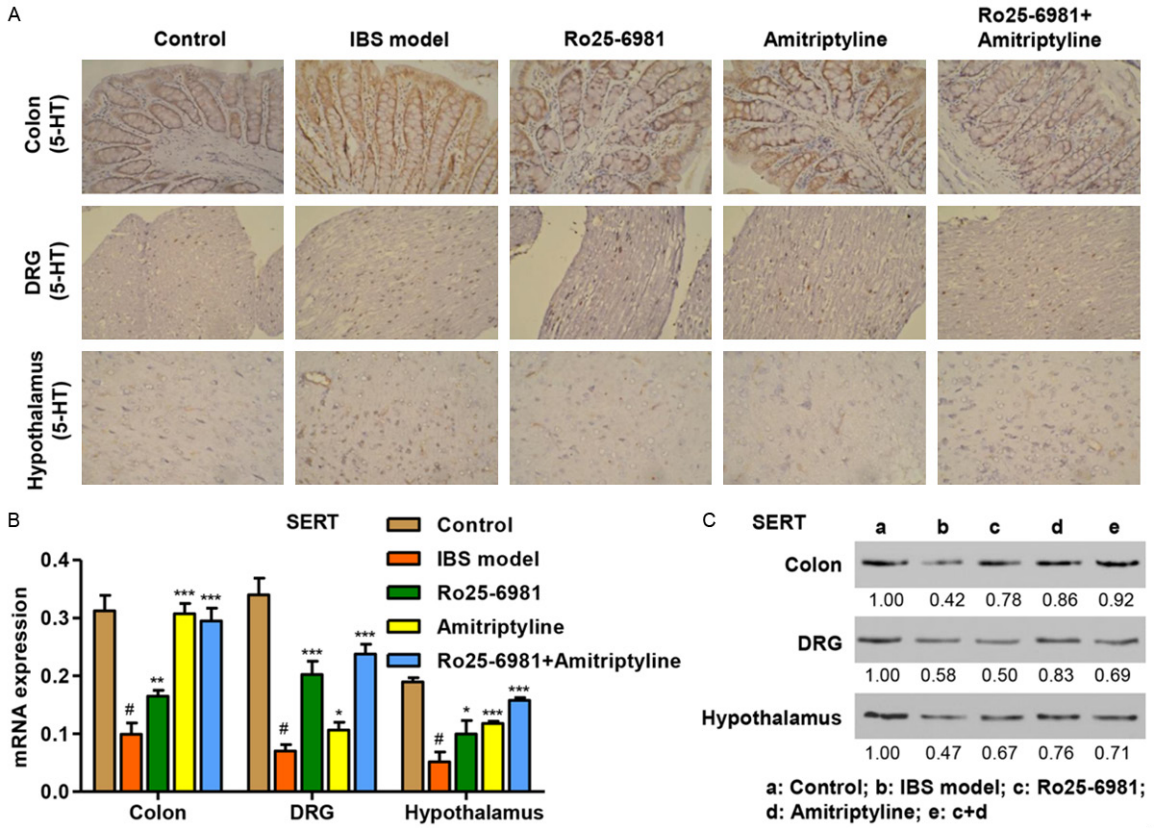

Figure 1. Expression of 5-HT and SERT in colon, DGR and hypothalamus in rats. (A) The expression of 5-HT in colon, DGR and hypothalamus was measured by immunohistochemical staining. The expression of SERT in colon, DGR and hypothalamus was measured by Real-Time Reverse Transcription-PCR (B) and Western blot analysis (C). #P<0.001 v.s. control; *P<0.05, **P<0.01, ***P<0.001 v.s. IBS model.

bit secondary antibody, developed using 3,3-diaminobenzidine (DAB) solution and counterstained with hematoxylin. To quantify 5-HT, NR2B, 5-HT2AR, 5-HT7R, TNF-α and IL-1β immunoreactivity, images were captured with a CDD spot camera mounted on a Nikon optical microscope at ×100 magnification.

Real-time reverse transcription-PCR

RNA was isolated by Trizol (Invitrogen, Paisley, UK). A total of 1 mg of RNA was reverse transcribed with the High Capacity cDNA Archive Kit (Applied Biosystems, Foster City, CA, USA). Real time-reverse transcription-PCR was performed by using Qiagen real-time RT-PCR kit (Hilden, Germany) according to the manufacturer's instructions. Primers were 5'-ATCTCCTAGAACCCTGTAAC-3' and 5'-GAAATGGACCTGGAGTATTG-3' for SERT; 5'-TCACGAAGTCCTGAGGTAAG-3' and 5'-AAGCTGGAGGGAAGCTAAAC-3' for NR2B; 5'-CCCTGCTCAATGTGTTTGTC-3' and 5'-ACTGTCTGCTCAGCATCTTC-3' for 5-HT2aR; 5'-CTTCAAGCGGGAACAGAAAG-3' and 5'-AGGAGGTGCCACAGATAAAG-3' for 5-HT7R; 5'-GTCGGTGTGAACGGATTTG-3' and 5'-TCCCATTCTCAGCCTTGAC-3' for GAPDH. The PCR reactions were carried out on a Bio-Rad real-time PCR detection system (Bio-Rad, Shanghai, China) by using 2 μg of synthesized cDNA. Target genes expression was normalized to the reference gene GAPDH. The $2^{-\Delta\Delta Ct}$ method was used to calculate relative gene expression.

Western blot analysis

Equal amounts of protein (80 μg) from the colon, DRG and hypothalamus of rats were separated and electro-transferred onto PVDF membranes (Invitrogen, USA), which were probed with goat anti-SERT monoclonal antibody (1:400, Santa cruz), mouse anti-NR2B monoclonal antibody (1:500, Abcam), goat anti-5-HT2AR monoclonal antibody (1:500, Santa cruz), rabbit anti-5-HT7R monoclonal antibody (1:500, Santa cruz) and mouse anti-GAPDH pri-

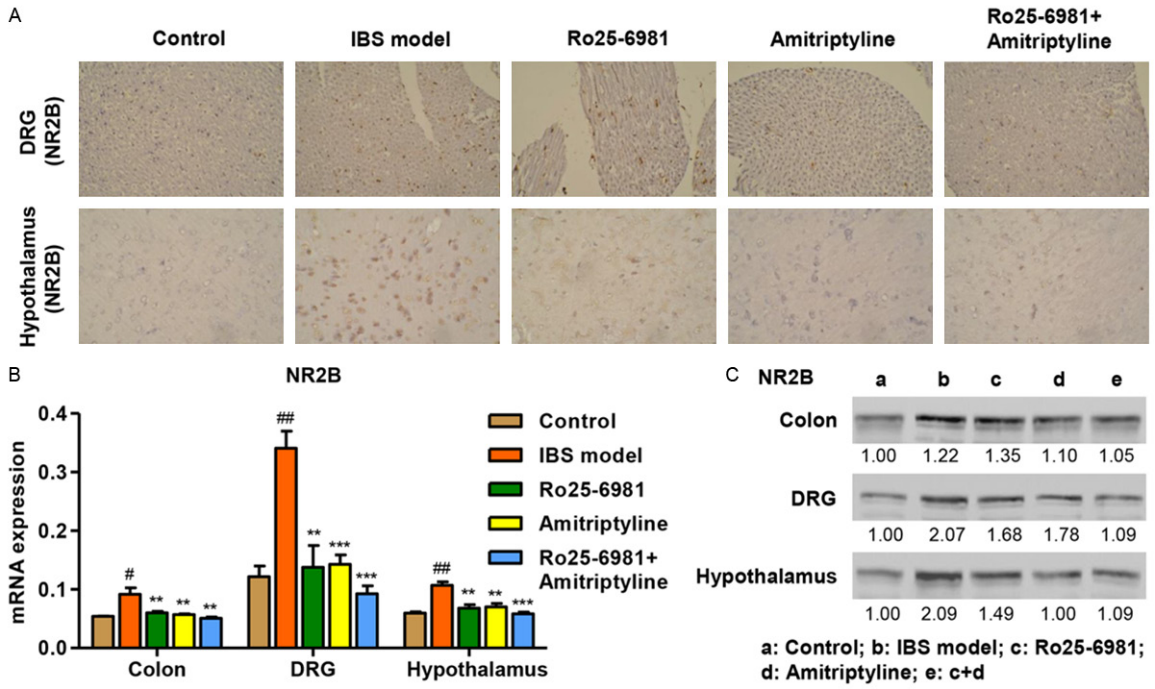

Figure 2. Expression of NR2B in colon, DGR and hypothalamus in rats. (A) The expression of NR2B in DGR and hypothalamus was measured by immunohistochemical staining. The expression of NR2B in colon, DGR and hypothalamus was measured by Real-Time Reverse Transcription-PCR (B) and Western blot analysis (C). #P<0.01, ##P<0.001 v.s. control; **P<0.01, ***P<0.001 v.s. IBS model.

mary antibody (1:1500, Cell Signaling Technology). Blots were washed and incubated in peroxidase-conjugated donkey anti-goat IgG (1:5000, Abcam), anti-rabbit IgG (1:5000, Abcam) or goat anti-mouse IgG (1:5000, Abcam). Blots were developed with the enhanced chemiluminescence method (ECL) following the manufacturer's instructions (Amersham).

Statistical analysis

Data are given as mean ± SD. The two-sided t-test or one-way ANOVA were applied to indicate significantly different mean values in comparison with the control group. A *P*-value o 0.05 was considered statistically significant.

Results

Expression of 5-HT and SERT in colon, DGR and hypothalamus

The immunohistochemical examination was focused on determining 5-HT expression in tissues of colon, DGR and hypothalamus. The number of 5-HT-positive cells in the colon, DGR and hypothalamus are shown in **Figure 1A**. The mean number of 5-HT-positive cells in the colon, DGR and hypothalamus was 76.6 ± 4.7, 61.7 ± 4.4 and 73.7 ± 1.1, respectively, in IBS model rats versus 55.2 ± 2.5, 42.8 ± 2.1 and 52.7 ± 3.0, respectively, in control rats. Moreover, the expression of 5-HT transporter protein (SERT) was also determined in colon, DGR and hypothalamus tissues. As shown in **Figure 1B**, the mRNA expression of SERT in the colon, DGR and hypothalamus was 0.09 ± 0.02, 0.07 ± 0.01 and 0.05 ± 0.02, respectively, in IBS model rats versus 0.31 ± 0.03, 0.34 ± 0.02 and 0.19 ± 0.01, respectively, in control rats. Similarly, the protein expression of SERT was also decreased in the colon, DGR and hypothalamus in IBS model rats compared with that in control rats (**Figure 1C**). These data suggest that IBS model increases 5-HT expression and decreases SERT expression in rats.

Effect of Ro25-6981 and amitriptyline on treatment on the expression of 5-HT and SERT in IBS model rats

Ro 25-6981, a selective NR2B antagonist, was intraperitoneal injected into rats, and amitriptyline, a 5-HT antagonist, was intragastric administrated rats, to demonstrate whether the NR2B

and 5-HT play a role in the development of IBS. The number of 5-HT-positive cells in the colon, DGR and hypothalamus response to Ro25-6981 and amitriptyline are shown in **Figure 1A**. The mean number of 5-HT-positive cells in the colon, DGR and hypothalamus was 67.4 ± 1.1, 50.4 ± 4.8 and 63.4 ± 3.8, respectively, in Ro25-6981 treated rats, and was 65.8 ± 6.1, 53.9 ± 4.4 and 61.0 ± 2.8, respectively, in amitriptyline treated rats, versus 76.6 ± 4.7, 61.7 ± 4.4 and 73.7 ± 1.1, respectively, in IBS model rats. Importantly, Ro25-6981 plus amitriptyline treatment significantly decreased the expression of 5-HT in the colon, DGR and hypothalamus by 11.9%, 13.5% and 13.7%, respectively, as compared with that in IBS model rats (**Figure 1A**).

Additionally, the mRNA expression of SERT in the colon, DGR and hypothalamus was significantly increased by 0.67-fold, 1.89-fold and 0.94-fold, respectively, in Ro25-6981 treated rats, and by 2.11-fold, 0.51-fold and 1.29-fold, respectively, in amitriptyline treated rats compared with that in IBS model rats (**Figure 1B**). Importantly, Ro25-6981 plus amitriptyline treatment significantly increased the mRNA expression of SERT in the colon, DGR and hypothalamus by 1.98-fold, 2.39-fold and 2.08-fold, respectively, as compared with that in IBS model rats (**Figure 1B**). Similarly, the protein expression of SERT was also increased in the colon, DGR and hypothalamus in Ro25-6981 and/or amitriptyline treated rats compared with that in IBS model rats (**Figure 1C**). These data suggest that Ro25-6981 and/or amitriptyline treatment decreases 5-HT expression and increases SERT expression induced by IBS in rats.

Expression of NR2B in colon, DGR and hypothalamus

The immunohistochemical examination was also focused on determining NR2B expression in tissues of DGR and hypothalamus. The number of NR2B-positive cells in the DGR and hypothalamus are shown in **Figure 2A**. The mean number of NR2B-positive cells in the DGR and hypothalamus was 65.9 ± 2.3 and 76.9 ± 2.2, respectively, in IBS model rats versus 42.5 ± 2.8 and 49.5 ± 4.3, respectively, in control rats. Moreover, the expression of NR2B was also determined in colon, DGR and hypothalamus tissues by Real-Time Reverse Transcription-PCR and Western blot analysis. As shown in **Figure 2B**, the mRNA expression of NR2B in the colon, DGR and hypothalamus was 0.09 ± 0.01, 0.34 ± 0.03 and 0.11 ± 0.01, respectively, in IBS model rats versus 0.05 ± 0.01, 0.12 ± 0.01 and 0.06 ± 0.01, respectively, in control rats. Similarly, the protein expression of NR2B was also increased in the colon, DGR and hypothalamus in IBS model rats compared with that in control rats (**Figure 2C**). These data suggest that IBS model induces increased NR2B expression in rats.

Ro25-6981 and amitriptyline on treatment attenuated expression of NR2B in IBS model rats

The number of NR2B-positive cells in the DGR and hypothalamus response to Ro25-6981 and amitriptyline are shown in **Figure 2A**. The mean number of NR2B-positive cells in the DGR and hypothalamus was 54.2 ± 5.1 and 68.0 ± 2.4, respectively, in Ro25-6981 treated rats, and was 49.6 ± 5.2 and 61.1 ± 1.9, respectively, in amitriptyline treated rats, versus 65.9 ± 2.3 and 76.9 ± 2.2, respectively, in IBS model rats. Importantly, Ro25-6981 plus amitriptyline treatment significantly decreased the expression of NR2B in the DGR and hypothalamus by 11.9% and 16.7%, respectively, as compared with that in IBS model rats (**Figure 2A**).

Additionally, the mRNA expression of NR2B in the colon, DGR and hypothalamus was significantly decreased by 34.1%, 59.7% and 37.3%, respectively, in Ro25-6981 treated rats, and by 38.5%, 58.2% and 34.6%, respectively, in amitriptyline treated rats compared with that in IBS model rats (**Figure 2B**). Importantly, Ro25-6981 plus amitriptyline treatment significantly decreased the mRNA expression of NR2B in the colon, DGR and hypothalamus by 44.4%, 73.5% and 45.8%, respectively, as compared with that in IBS model rats (**Figure 2B**). Similarly, the protein expression of NR2B was also decreased in the colon, DGR and hypothalamus in Ro25-6981 and/or amitriptyline treated rats compared with that in IBS model rats (**Figure 2C**). These data suggest that Ro25-6981 and/or amitriptyline treatment decreases NR2B expression induced by IBS in rats.

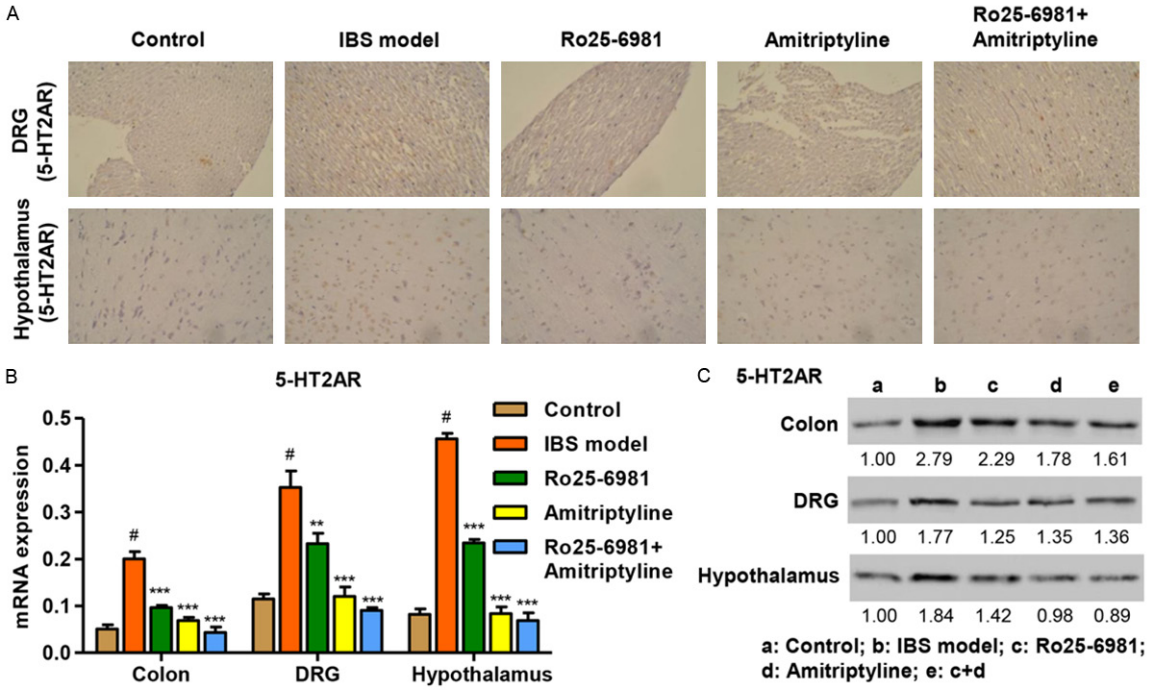

Figure 3. Expression of 5-HT2AR in colon, DGR and hypothalamus in rats. (A) The expression of 5-HT2AR in DGR and hypothalamus was measured by immunohistochemical staining. The expression of 5-HT2AR in colon, DGR and hypothalamus was measured by Real-Time Reverse Transcription-PCR (B) and Western blot analysis (C). #P<0.001 v.s. control; **P<0.01, ***P<0.001 v.s. IBS model.

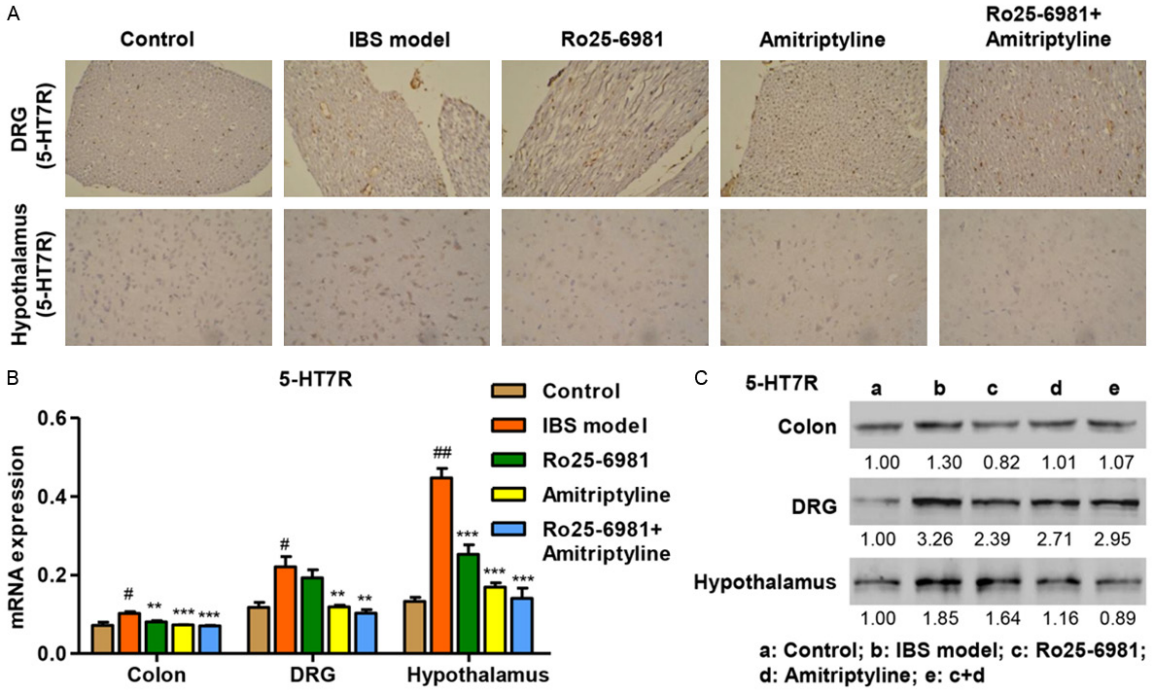

Figure 4. Expression of 5-HT7R in colon, DGR and hypothalamus in rats. (A) The expression of 5-HT7R in DGR and hypothalamus was measured by immunohistochemical staining. The expression of 5-HT7R in colon, DGR and hypothalamus was measured by Real-Time Reverse Transcription-PCR (B) and Western blot analysis (C). #P<0.01, ##P<0.001 v.s. control; **P<0.01, ***P<0.001 v.s. IBS model.

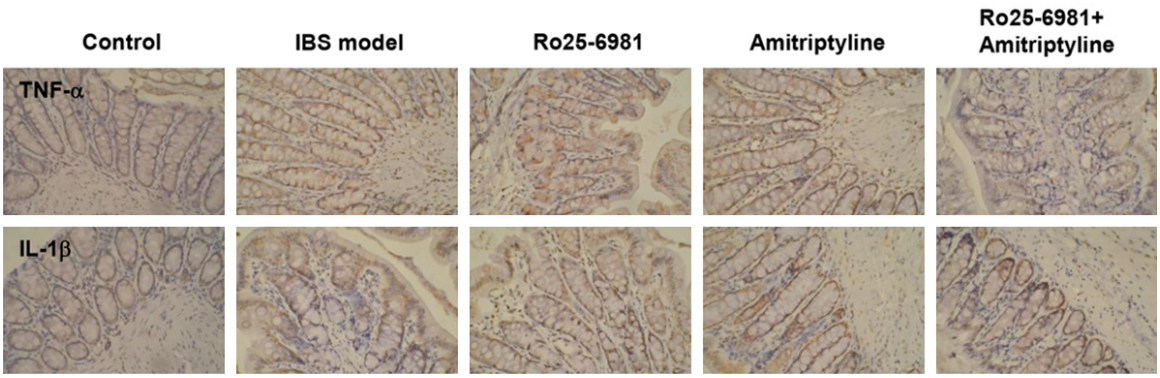

Figure 5. Expression of TNF-α and IL-1β in colon tissues in rats. The expression of TNF-α and IL-1β in colon tissues was measured by immunohistochemical staining.

Expression of 5-HT2AR and 5-HT7R in colon, DGR and hypothalamus

The immunohistochemical examination was also focused on determining 5-HT2AR and 5-HT7R expression in tissues of DGR and hypothalamus. The number of 5-HT2AR-positive cells in the DGR and hypothalamus are shown in **Figure 3A**. The mean number of 5-HT2AR-positive cells in the DGR and hypothalamus was 67.6 ± 2.7 and 76.4 ± 3.5, respectively, in IBS model rats versus 45.5 ± 4.2 and 62.2 ± 3.3, respectively, in control rats. Moreover, the expression of 5-HT2AR was also determined in colon, DGR and hypothalamus tissues by Real-Time Reverse Transcription-PCR and Western blot analysis. As shown in **Figure 3B**, the mRNA expression of 5-HT2AR in the colon, DGR and hypothalamus was increased by 2.94-fold, 2.07-fold and 4.57-fold, respectively, in IBS model rats versus that in control rats. Similarly, the protein expression of 5-HT2AR was also increased in the colon, DGR and hypothalamus in IBS model rats compared with that in control rats (**Figure 3C**).

Additionally, the number of 5-HT7R-positive cells in the DGR and hypothalamus are shown in **Figure 4A**. The mean number of 5-HT7R-positive cells in the DGR and hypothalamus was 61.7 ± 3.7 and 72.8 ± 5.3, respectively, in IBS model rats versus 46.4 ± 6.1 and 66.1 ± 2.8, respectively, in control rats. Moreover, the expression of 5-HT7R was also determined in colon, DGR and hypothalamus tissues by Real-Time Reverse Transcription-PCR and Western blot analysis. As shown in **Figure 4B**, the mRNA expression of 5-HT7R in the colon, DGR and hypothalamus was increased by 0.44-fold, 0.88-fold and 2.37-fold, respectively, in IBS model rats versus that in control rats. Similarly, the protein expression of 5-HT7R was also increased in the colon, DGR and hypothalamus in IBS model rats compared with that in control rats (**Figure 4C**). These data suggest that IBS model induces increased 5-HT2AR and 5-HT7R expression in rats.

Ro25-6981 and amitriptyline on treatment attenuated expression of 5-HT2AR and 5-HT7R in IBS model rats

The number of 5-HT2AR-positive and 5-HT7R-positive cells in the DGR and hypothalamus response to Ro25-6981 and amitriptyline are shown in **Figures 3A** and **4A**. The mean number of 5-HT2AR-positive cells in the DGR and hypothalamus was 52.7 ± 4.6 and 68.3 ± 4.1, respectively, in Ro25-6981 treated rats, and was 53.7 ± 5.3 and 70.3 ± 2.9, respectively, in amitriptyline treated rats, versus 67.6 ± 2.7 and 76.4 ± 3.5, respectively, in IBS model rats (**Figure 3A**). The mean number of 5-HT7R-positive cells in the DGR and hypothalamus was 59.2 ± 9.7 and 70.2 ± 2.6, respectively, in Ro25-6981 treated rats, and was 54.0 ± 5.2 and 68.5 ± 1.2, respectively, in amitriptyline treated rats, versus 61.7 ± 3.7 and 72.8 ± 5.3, respectively, in IBS model rats (**Figure 4A**). Importantly, Ro25-6981 plus amitriptyline treatment significantly decreased the expression of 5-HT2AR in the DGR and hypothalamus by 12.8% and 8.2%, and decreased the expression of 5-HT7R in the DGR and hypothalamus by 5.8% and 10.3%, respectively, as compared with that in IBS model rats (**Figures 3A** and **4A**).

Additionally, the mRNA expression of 5-HT2AR in the colon, DGR and hypothalamus was significantly decreased by 52.1%, 34.1% and 48.7%, respectively, in Ro25-6981 treated rats, and by 65.8%, 65.7% and 81.7%, respectively, in amitriptyline treated rats compared with that in IBS model rats (**Figure 3B**). The mRNA expression of 5-HT7R in the colon, DGR and hypothalamus was significantly decreased by 21.2%, 12.6% and 43.5%, respectively, in Ro25-6981 treated rats, and by 28.7%, 46.1% and 62.2%, respectively, in amitriptyline treated rats compared with that in IBS model rats (**Figure 4B**). Importantly, Ro25-6981 plus amitriptyline treatment significantly decreased the mRNA expression of 5-HT2AR in the colon, DGR and hypothalamus by 78.5%, 74.4% and 85.1% (**Figure 3B**), and decreased the expression of 5-HT7R in the DGR and hypothalamus by 30.8%, 53.3% and 68.5%, respectively, as compared with that in IBS model rats (**Figure 4B**). Similarly, the protein expression of 5-HT2AR and 5-HT7R was also decreased in the colon, DGR and hypothalamus in Ro25-6981 and/or amitriptyline treated rats compared with that in IBS model rats (**Figures 3C** and **4C**). These data suggest that Ro25-6981 and/or amitriptyline treatment decreases 5-HT2AR and 5-HT7R expression induced by IBS in rats.

Ro25-6981 and amitriptyline on treatment attenuated expression of TNF-α and IL-1β in IBS model rats

The immunohistochemical examination was focused on determining TNF-α and IL-1β expression in tissues of colon. The number of TNF-α-positive and IL-1β-positive cells in the colon is shown in **Figure 5**. The mean number of TNF-α-positive and IL-1β-positive cells in the colon was 74.8 ± 1.7 and 75.7 ± 5.9, respectively, in IBS model rats versus 53.6 ± 3.1 and 50.8 ± 3.9, respectively, in control rats. The number of TNF-α-positive and IL-1β-positive cells in the colon response to Ro25-6981 and amitriptyline are also shown in **Figure 5**. The mean number of TNF-α-positive and IL-1β-positive cells in the colon was 61.7 ± 4.6 and 64.2 ± 4.1, respectively, in Ro25-6981 treated rats, and was 67.6 ± 1.5 and 64.8 ± 2.9, respectively, in amitriptyline treated rats, versus 74.8 ± 1.7 and 75.7 ± 5.9, respectively, in IBS model rats. Importantly, Ro25-6981 plus amitriptyline treatment significantly decreased the expression of TNF-α and IL-1β in the colon by 9.5% and 19.7%, respectively, as compared with that in IBS model rats (**Figure 5**). These data suggest that Ro25-6981 and/or amitriptyline treatment decreases TNF-α and IL-1β expression induced by IBS in rats.

Discussion

Irritable bowel syndrome (IBS) is primarily caused by central sensitization, due to the vulnerability and susceptibility of the neonatal nervous system to plastic changes, has been shown in animal models [19]. In this study, we established a rat model of IBS using the procedure reported by Al-Chaer et al. [18] whereby neonatal rats received acetic acid stumuli. Using the rats which developed IBS as a visceral hyperalgesia model, we found that the model rats showed higher expression of 5-HT, NR2B, 5-HT2AR and 5-HT7R in colon, DGR and hypothalamus than that in the normal control rats. The selective antagonist of NR2B and 5-HT, Ro25-6981 and amitriptyline, were significantly decreased the expression of 5-HT, NR2B, 5-HT2AR and 5-HT7R. This result indicated that 5-HT and NR2B may play important role in the brain-gut axis in the development of IBS in rats.

Recent therapeutic successes with 5-HT-modulating agents have encouraged a re-examination of the role of 5-HT in IBS. 5-HT3 antagonists decrease urgency and frequency in diarrhea-predominant IBS and improve stool consistency [20], whereas 5-HT4 agonists stimulate bowel frequency and decrease stool consistency [15]. In this study, increased 5-HT and decreased SERT expression in colon, DGR and hypothalamus was found in IBS model, while amitriptyline treatment corrected them. Amitriptyline is a complex drug, exerting a range of pharmacological effects including inhibition of noradrenaline, 5-HT and adenosine uptake [21]. Similar to our findings that constipation-predominant-IBS but not diarrhea-predominant-IBS patients had increased platelet 5-HT level and were both higher compared with healthy controls [22]. Under normal circumstances, SERT terminates 5-HT action via uptake into enterocytes and serotonergic neurons. Indeed, other study has shown reduced mRNA expression of SERT in rectal mucosa, which increases the availability of 5-HT in IBS patients, whether associated with diarrhea or constipation [23].

Central sensitization is an important mechanism underlying IBS. Previous studies have revealed that central sensitization of somatic pain was mediated by spinal NMDA receptor, especially NR2B subunit [24]. Expressions of spinal NR2B subunit significantly increased in IBS-like rats when compared with that in control rats, and was inhibited by Ro25-6981, the NR2B subunit antagonist [25], which in line with our results. Furthermore, Ro25-6981 could dose-dependently attenuate visceral pain in IBS-like rats. Therefore, we can infer reasonably that spinal cord NR2B subunit is also responsible for the central sensitization in visceral pain, similar to inflammatory and neuropathic pain [26].

5-HT2A receptor (5-HT2AR) activation mediates secretory responses in human colonic mucosa and the agonists of 5-HT2AR should increase transit in humans with disorders of reduced gastrointestinal transit including IBS [27]. 5-HT7 receptor (5-HT7R) plays a role in smooth muscle relaxation in a variety of tissues and so it might be involved in diseases such as IBS [28]. Inhibiting 5-HT7R causes the elevation of pressure threshold which results in blockage of stimulating intestinal peristalsis and reduction of bowel compliance [29]. In the present study, the expression of 5-HT2AR and 5-HT7R was significant high in colon, DGR and hypothalamus in IBS model compared with control. However, Ro25-6981 and/or amitriptyline treatment inhibited increased expression of 5-HT2AR and 5-HT7R in IBS rats.

Most investigations showed the immunity alteration in IBS patients with significantly higher level of proinflammatory cytokines (IL-1β, IL-2, IL-6, IL-8, IL-12, IL-18, TNF-α, IFN-γ) and lower level of anti-inflammatory cytokines (IL-4, IL-1082), whether in peripheral blood or in intestinal mucosa [11, 30]. Secretion of IL-1β from colonic CD3+/CD28+ T lymphocytes correlated modestly with bowel habit dissatisfaction in adults with IBS [31]. And depression scores were found to have higher TNF-α production in peripheral blood mononuclear cells associated with IBS [32]. Our results showed that TNF-α and IL-1β were overexpressed in colon of IBS model rats compared with control rats, and Ro25-6981 and/or amitriptyline treatment inhibited TNF-α and IL-1β upregulation significantly. Moreover, low grade inflammation-immune activation has been suggested to be one of the most important mechanisms of brain-gut interaction, which has been resulted from the influence of TNF-α and IL-1β in the development of visceral pain and psychological disorders, mediated by the central nervous system [33]. Thus, Ro25-6981 and amitriptyline, demonstrated to be a high-efficiency candidate for inhibition of 5-HT, NR2B, 5-HT2AR and 5-HT7R as well as the proinflammatory cytokines in IBS rats.

In conclusion, our results imply that 5-HT, SERT, NR2B, 5-HT2AR, 5-HT7R, TNF-α and IL-1β in the colon, DGR and hypothalamus are involved in the development of visceral hyperalgesia in rats induced by acetic acid stumuli. Ro25-6981 and amitriptyline can attenuate such visceral hyperalgesia and may through downregulation of the 5-HT and NR2B.

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Disclosure of conflict of interest

None.

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