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Research Paper

The role of 5-HT$_7$ Receptor in the pathogenesis of IBS ☆

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Abstract

Objective: To investigate the role of 5-HT$_7$ receptor in the pathogenesis of irritable bowel syndrome(IBS). **Methods:**Rat model of D-IBS was established by intracolonic instillation of acetic acid and restraint stress; Rat model of C-IBS was established by stomach irrigated with 0-4°C cool water daily for 14 d. The content and distribution of 5-HT$_7$ receptor at the brain and bowel was examined by immunohistochemistry and the expression of 5-HT$_7$ receptor mRNA was detected by fluorescence quantitative RT-PCR(Real-time PCR). **Results:**Immunocytochemistry result showed the 5-HT$_7$ rceptor positive staining at hippocampus and hypothalamus of both C-IBS and D-IBS group was stronger than that of control group($P < 0.01$). The 5-HT$_7$R expression at ileum, proximate colon, distal colon of C-IBS group was significantly stronger than that of control group($P < 0.05$). Realtime-PCR analysis results showed the expression level of 5-HT$_7$ receptor at hippocampus and hypothalamus of both C-IBS and D-IBS group was increased than that of control group($P < 0.05$). At proximal and distal colon of C-IBS group, the 5-HT$_7$ receptor mRNA expression was increased compared with control group($P < 0.05$). **Conclusion:**The up-regulated expression of 5-HT$_7$ receptor at brain and colon may play an important role in the pathogenesis of C-IBS.

Key words: 5-hydroxytryptamine 7 receptor; diarrhea-predominant IBS; constipation-predominant IBS; pathogenesis

INTRODUCTION

Irritable Bowel Syndrome(IBS) is a complicated disease correlated with gastroenterological motility, neuroendocrine and visceral sensation. According to the clinical symptom, it is mainly divided into two subgroups:Diarrhea-predominant Irritable Bowel Syndrome(D-IBS) and Constipation-predominant Irritable Bowel Syndrome(C-IBS). 5-hydroxytryptamine (5-HT) is an important neurotransmitter(NT) regulating the function of gut. 5-HT and its receptors were extensively distributed in the central nerve system(CNS), peripheral nerveous system and gastrointestinal tract (GIT), participating in regulating the functions of psychology, nerve and gut. The biological effect of 5-HT was activated by combining with different receptors located at all the effective tissues and cells. It was known until now that there were 7 types of 5-HT receptor(5-HT$_{1\text{-}7}$R). 5-HT$_7$R, a G-protein-coupling receptor which has been found recently (having four subtype at least[1]) plays a role in regulating smooth muscle relaxatant of gastrointestine and peripheral nociceptive pathways. 5-HT$_7$R may be involved in the mechanism of the gastrointestinal dyskinesia, abdominal pain and visceral paresthesia in IBS, however its role in the pathogenesis of IBS hasn' t been reported. Our study aims to investigate 5-HT$_7$ role in the pathogenesis of IBS by observing the difference of 5-HT$_7$R expression at brain and gut in each subtype of IBS.

METHODS AND MATERIAL

Animal model and experiment groups

Adult Sprague-Dawley rats, 220 g to 250 g, were obtained from the Animal Supply Centre of Xi' an Jiaotong University. All procedures were in accordance with the guidelines of Xi' an Jiaotong University

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Animal Care and Use Committee. The rats were randomly separated into 3 groups (every group containing 15 rats): A control group, C-IBS group and D-IBS group. Rats of C-IBS group were pretreated with 0~4℃ cool water(2 ml) to irrigate the stomach daily for 14 d[2]. After fasting for 12 h, rats of D-IBS group were anesthetized by inhalating ether, and then a silica gel pipe was insert into the colon about 8 cm to the anus. 4% acetic acid (1 ml) was instilled into the colon through the pipe for 30 s. The colon was then flushed with 2 ml PBS buffer. After 6 days of recovery, their forelegs were constrainted to move for 3 h[3].The control group didn't receive interference. All rats were maintained under controlled temperature(21~24℃) and light (lights on 8:00-20:00) conditions with free access to water and laboratory chow pellets.

Tissue preparation

8 rats of each group were deeply anesthetized with chloralhydrate(2 ml/kg ip) and perfused intracardially with cold normal saline 300 ml followed by 4% p-formaldehyde in 0.1 mol/L sodium phosphate buffer (pH 7.4). The brain tissue, 2 cm segment of jejunum, ileum, proximal colon and distal colon were removed, postfixed for 12 h in the fixative and immersed in 25% sucrose until the tissue sank to the bottom. All tissues were cut with a cryostat at −20℃ and mounted on gel-coated slides. 7 rats of each group were deeply anesthetized with chloralhydrate, and then hippocampus, hypothalamus, jejunum, ileum, proximal colon and distal colon were removed carefully without RNASE to examine mRNA by realtime-PCR.

Immunohistochemical staining for the 5-HT$_7$ receptor

Staining was performed according to the instructions of the kit(immunohistochemical ABC method). The primary antibody was rabbit anti rat 5-HT$_7$ antibody (Calbiochem Bioscience, inc, Germany) and the secondary antibody was biotin-labelled sheep anti-rabbit IgG(Boster Bio-project Co. Wuhan, China).Tissue sections were incubated at 4℃ for 24 h in the primary antibodies(1:300 dilution), and then incubated at room temperature for 1h and ABC complex(Boster Bio-project Co. Wuhan, China) incubated 30min.The slides were washed in water to stop the reaction, then dehydrated in alcohol, cleared in xylene and covered slide with cover slip and gum. Every slide was assayed for the staining brightness at random 5 visual field. Phophate buffered saline(PBS) substituted for the first antibody as negative control.

Determination of 5-HT$_7$ receptor mRNA

Total mRNA was extracted from all tissue using the Trizol reagent(Invitrogen, USA) according to the anufacturer's instructions.1.5 μg of total RNA was used for reverse transcription using ExSccriptTM RT reagent Kit(TaKaRa Biotechnology Co, Dalian). Real Time RT-PCR was performed with Master mix (TOYOBO COLTD, Japan) following the manufacturer's protocol. Amplification using the following primers: 5-HT$_7$R(designed as Genebank Accession# NM 022938) forward primer:5'-GCT CAT CAC GCT GCT GAC GAT-3'; reverse primer:5'-CGC CAG GGA CAC AAT CAG G-3'. The legth of objective product was 106 bp; GAPDH primer(designed as Genenbank Accession #NM 17701) was used as control: forward primer:5' CAG TGC CAG CCT CGT CTC ATA 3'; reverse primer:5' TGC CGT GGG TAG AGT CAT A 3'. The length of product was 184 bp. Real Time RT-PCR was performd for 40 cycles with ABI prism 7000(ABI,US):95℃ for 60 s, denaturing at 95℃ for 15 s, annealing at 58℃ for 16 s and elongating at 72℃ for 45 s, with a final elongation at 72℃ for 5 min. GAPDH and 5-HT$_7$R of each sample were amplified with same condition and every reaction free control was designed(reacting system without template) to eliminate contamination. The relative minimum cycle number ($2^{\blacktriangle CT}$) was used[4] to calculate the comparative gene copy of 5-HT$_7$R:$2^{Ct(GAPDH)-Ct(5\text{-}HT_7R)}$.

Statistical analysis

All data were expressed as mean±standard deviation (SD). The significance of differences was analyzed by couple T test and One-way ANOVA using SPSS 10. 0. *P*-value < 0.05 was considered the significant difference.

RESULTS

Immunohistochemical staining for the 5-HT$_7$ receptor in brain and intestine

5-HT$_7$ positive cells extensively exited at cortex, hippocampus, thalamus and hypothalamus in brain, submucosa neuroplexus and intermuscular neuroplexus in gut. The result of 5-HT$_7$ expression at hippocampus and hypothalamus of both C-IBS and D-IBS were significant stronger than that of control($P < 0.01$)(the value of brightness opposite to the expression quantity). At ileum, proximal colon and distal colon in C-IBS group, 5-HT$_7$ R expression was stronger than that of control($P < 0.05$)(***Tab1 Fig1,2***).

Epression of 5-HT$_7$ receptor in difference subgroup of IBS model

Because quality of RNA affects the result greatly, RNA was detect with spectrophotometer and the value 260/280 were above 1.80. By electrophoresed in 1.0% agarose gel, the products' strap of realtime RT-PCR

Tab 1 **The comparision of 5-HT$_7$ receptor immunohistochemical staining brightness** ($\bar{x} \pm s$, $n = 8$)

Group	Hippocampus(CA3)	Hypothalamus	Jejunum	Ileum	Proximal colon	Distal colon
C-IBS	146.3 ± 7.1$^{\#\bigstar}$	144.7 ± 9.5$^{\#}$	142.3 ± 8.6	133.2 ± 14.1$^{\#}$	142.6 ± 9.1$^{\blacktriangle}$	144.9 ± 6.0$^{\#}$
D-IBS	153.0 ± 4.9$^{\#}$	153.8 ± 8.5$^{\#}$	136.9 ± 7.9	146.7 ± 14.5	153.9 ± 11.7	165.7 ± 8.3
control	170.4 ± 3.69	171.3 ± 8.9	146.5 ± 2.1	156.6 ± 9.8	159.8 ± 8.8	157.5 ± 10.7

Compared with control group,$^{\blacktriangle}$ $P < 0.05$, $^{\#}P < 0.01$; Compared with D-IBS group, $^{\bigstar}$ $P < 0.05$.

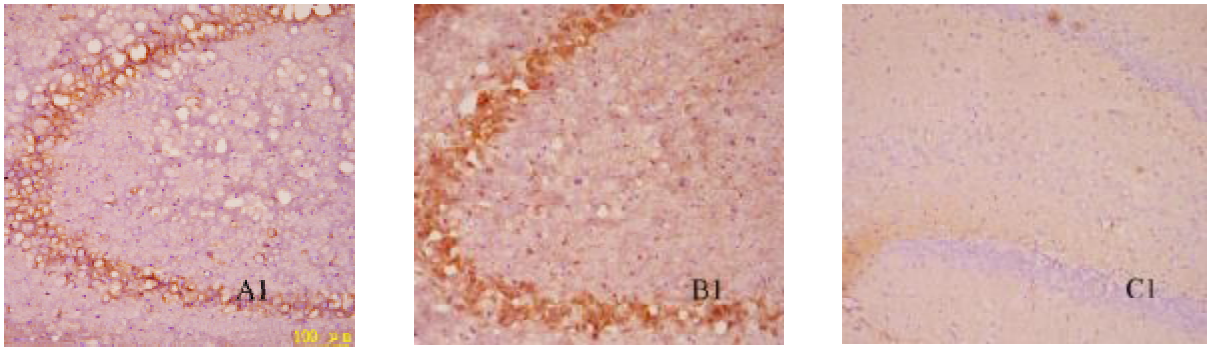


Fig 1 5-HT$_7$ receptor staining at hippocampus in each group(× 200, A: C-IBS; B: D-IBS; C :control)

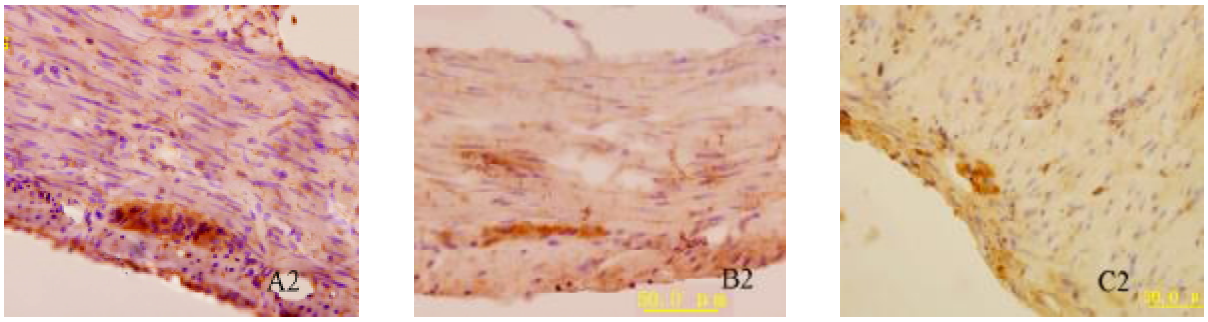


Fig 2 5-HT$_7$ receptor staining at distal colon in each group(× 400, A: C-IBS; B: D-IBS; C: control)

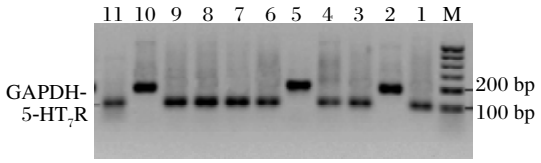
were distinct(106 bp and 184 bp) and consistent with prediction of primer. The expression of 5-HT$_7$R was enhanced in the hippocampus and hypothalamus in both C-IBS and D-IBS.

compared with the control group($P < 0.05$). The expression of 5-HT$_7$R atileum, proximal colon and distal colon in C-IBS was higher than that in control($P < 0.05$).There was no difference at gut between D-IBS group and control(***Tab 2, Fig3,4***).

Tab 2 **The comparison of 5-HT$_7$ receptor mRNA copies in each group** ($\bar{x} \pm s$, $n = 8$)

Group	Hippocampus(× 10^{-3})	Hypothalamus(× 10^{-3})	Jejunum(× 10^{-3})	Ileum(× 10^{-3})	Proximal colon(× 10^{-3})	Distal colon(× 10^{-3})
C-IBS	15.75 ± 7.08$^{\blacktriangle}$	16.46 ± 8.23$^{\blacktriangle}$	29.42 ± 12.50	34.07 ± 15.99$^{\blacktriangle}$	18.79 ± 10.57$^{\blacktriangle}$	17.85 ± 10.5$^{\blacktriangle\bigstar}$
D-IBS	16.32 ± 6.43$^{\blacktriangle}$	15.93 ± 3.54$^{\blacktriangle}$	22.83 ± 11.27	24.27 ± 8.59	9.05 ± 4.15	10.97 ± 4.05
control	8.77 ± 3.59	9.49 ± 3.59	26.44 ± 8.19	18.01 ± 8.81	9.89 ± 5.48	9.11 ± 5.01

Compared with control,$^{\blacktriangle}$ $P < 0.05$; Compared with D-IBS, $^{\bigstar}$ $P < 0.05$.



1-4: distal colon, proximal colon, ileum and jejunum of control group; 5-8: distal colon, proximal colon, ileum and jejunum of C-IBS; 9-11: hippocampus of C-IBS, D-IBS and control group.

Fig 3 Electrophoresis picture of 5-HT$_7$R and GAPDH

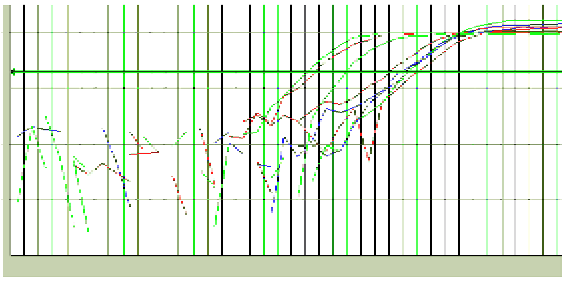


Fig 4 GAPD and 5-HT$_7$ receptor amplification curve

DISCUSSION

Irritable bowel syndrome(IBS) is present in 10% to 22% of the general population and is the most common cause of visiting gastroenterologists(28%). The pathological mechanism of IBS was not still completely clear. It was recognized that gastreointestinal dyskinesis, visceral paresthesia and gut abnormal secretion were due to the cardinal pathological mechanism, and the dysfunction of brain-gut reaction, inflammation, abnormal regulation of nerve-immunity-secretion had an important role in the morbility of IBS. 5-HT, as an important neurotransmitter(NT), has many physiological functions. Recently research found that intestinal mucosa caused gut peristalsising and evoked 5-HT releasing while 5-HT agitated its receptor to provocate peristalsising and regulating intestinal secrete[5,6].

The 5-HT_7 receptor exits in the diverse organisms of humans and animals, and has multiple functions, such as body temperature regulation, REM sleep modulation and the circadian timing system. There was a report that subtypes of 5-HT_7R distributed differently in CNS and gut, and their expression level and distribution in gut were different[7,8]. 5-HT_7R was found early in effective cells in bowel. It can regulate the smooth muscle relaxion of human colon[9], guinea-pig ileum[10], proximal gastric relaxation of awake dogs[11] and restrain gut peristalsis[12]. Blocking 5-HT_7R may decrease the threshold pressure volume of evoking enterokinesia[13]. Whether there are differences of 5-HT_7R expression in the brains and guts of IBS is unknown and the role of 5-HT_7R in IBS occurrence has not been reported.

Our results showed the expression of 5-HT_7R in the hippocamps and hypothalamus of C-IBS and D-IBS increased in comparison with control group. In C-BS proximal and distal colon, the expression of 5-HT_7 receptor was higher than that of control. There was no difference at gut between D-IBS and control group. 5-HT_7 receptor is the most recently identified member of the family of G-protein-coupled 5-HT receptors. Its stimulation caused Gsa-mediated adenylate cyclase1,5, 8 action[14]. Known adenylate cyclase isoforms are sensitive to Gsa stimulation *in vitro*. However, the Ca^{2+} calmodulin-stimulated isoforms adenylate cyclase 1 and adenylate cyclase 8 were insensitive to Gsa *in vivo*. Activation of adenylate cyclase1 and adenylate cyclase 8 were particularly intriguing because these isoforms were neural specific[15] and expressed in areas of the brain where 5-HT_7 receptors localized, such as the hippocampus and hypothalamus. This suggested that 5-HT might regulate cAMP in the certain areas of the brain by mobilizing intracellular Ca^{2+} after activating 5-$HT_{7(a)}$ receptors. There was also some evidence that 5-HT_7 receptors play a role in mediating stress and glucocorticoid-induced effects on hippocampal neurogenesis which had been implicated in depression[16]. Blomhoff [17] research demonstrated forebrain information processing related to viscera sensitivity of IBS patients. Forebrain hyperactivity found with anxiety patients might be linked to the psychology-biological mechanism that IBS patients with mental disorders experience and the severity of symptoms and the resultant course of the disease. So the high expression of 5-HT_7R in the brain might be related to mental symptoms and viscera hypersensitivity. Marcello Tonini's study[18] provided evidence of 5-HT_7 receptors expression on AH neurons and on descending neuron containing nNOS/VIP, and that endogenous 5-HT modulates threshold pressure for peristslsis and circular muscle accommodation through the activation of these receptors. Muscular 5-HT_7 receptors, which were present on the circular muscle layer, may also participate in the accommodation process through a direct muscle relaxant effect(the 5-HT_7 receptor can couple adenyl cyclase and regulate cAMP synthesizing). Marcello Tonini hypothesized that, under pathophysiologic conditions, overstimulation of these receptors might cause an exaggerated accommodation of the circular muscle that could contribute to abdominal bowl disorders. Our study showed the expression of 5-HT_7 receptor in C-IBS colon was upregulated, and it might be an important pathophysiologic mechanism in C-IBS occurrence and be related to the abnormal colon motility. Further human studies will be needed to test this hypothesis.

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