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Research Article

Effect of 5-hydroxytryptamine Receptor in the Lower Esophageal Sphincter Regulation Mechanism

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Abstract: As a neurotransmitter and avascular active substance, the 5-hydroxytryptamine (5-HT, serotonin) is widely distributed in the central nervous system and surrounding tissues. The 5-HT can play its role by acting on its corresponding 5-HT receptor. Nowadays, the 5-HT receptor can be classified into seven, according to different signal transduction method of receptors, the 5-HT3 receptor belongs to the ligand-gated ion channels, while other six 5-HT receptors are involved into the G protein-coupled receptors and play the biological role by binding to specific G protein-coupled receptors (GPCRs) on the surface of the cell membrane. The 5-HT plays an important role in the brain-gut information transmission and studies showed that the physiological stimulations like having meals, and pathological stimulations like ischemia and stress could promote the release of the 5-HT. In the gastrointestinal tract, the 5-HT is closely related to gastrointestinal sensitivity, gastrointestinal movement and secretion regulation, as well as many gastrointestinal dysfunction disorders, such as gastrointestinal power and visceral sensitivity abnormality and abnormalities of brain-gut axis.

Keywords: 5-serotonin; Lower esophageal sphincter; Neurotransmitter

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1 Introduction

The 5-HT plays an important role in the brain-gut information transmission and studies showed that the physiological stimulations like having meals, and pathological stimulations like ischemia and stress could promote the release of the 5-HT[1-3]. In the gastrointestinal tract, the 5-HT is closely related to gastrointestinal sensitivity, gastrointestinal movement and secretion regulation, as well as many gastrointestinal dysfunction disorders, such as gastrointestinal power and visceral sensitivity abnormality and abnormalities of brain-gut axis[4-6].

In the first part of this project, reverse transcriptase-polymerase chain reaction (RT-PCR), real-time quantitative PCR and Western-blot were used to detect the expression of seven 5-HT receptor sub-type mRNAs and protein in the clasp and sling fibres of the lower esophageal sphincter (the LES), and compared with the expression of the smooth muscles of esophageal and gastric fundus. Studies have shown that seven 5-HT receptor sub-type mRNAs were expressed in the clasp and sling fibres of the LES, including the 5-HT1AR, 5-HT2AR, 5-HT3AR, 5-HT4R, 5-HT5AR, 5-HT6R and 5-HT7R, in which the level of the 5-HT3A and 5-HT4 receptor mRNAs was the highest. The Western-blot was used to confirm that five 5-HT receptor subtypes were expressed in the clasp and sling fibres of the LES, including the 5-HT1AR, 5-HT2AR, 5-HT3AR, 5-HT4R, 5-HT5AR, 5-HT6R and 5-HT7R,

in which the level of the 5-HT3A and 5-HT4 receptor mRNAs was the highest. There are differences in the expression of different receptor subtype mRNAs and its corresponding proteins in the same muscle strip, while no difference exists in the expression of same receptor subtype mRNAs.

Esophageal motility disorders, gastroesophageal reflux disease, achalasia and diffuse esophageal motility disorders, were often associated with the dysfunction of the LES, and mainly manifested by not fully entering the stomach of foods or abnormal reflux of foods and stomach fluid. In clinical practice, calcium channel blockers, nitric oxide donor, botulinus toxin as well as surgeries were often used to treat, but the effect was poor[7]. Nowadays, it has been verified that the 5-HT receptor is closely related to gastrointestinal functions, for example, the 5-HT3 receptor antagonists were often used for anti-emesis, while the 5-HT4 receptor agonists were used to promote the gastrointestinal peristalsis, but there are few studies on the effects of the 5-Ht receptor in the LES.

To further confirm the effect of the 5-HT receptor in the regulation of the LES' contraction and relaxation, this part of the experimental study plans to use the isolated muscle tension measurement technology to detect the effects of the receptor agonists and antagonists of the non-selective and selective 5-HT on the clasp and sling fibres of the LES, and preliminarily explores the effects of the 5-HT receptor in the regulation of the LES' functions to lay a foundation for the further research.

2 Research Objective

28 patients, including 16 male patients and 12 female patients, averagely aged 58 with subtotal gastrectomy and digestive tract reconstruction surgery for thoracic middle segment esophageal carcinoma in the Thoracic Surgery Department, the Affiliated Hospital of Hebei University during the period from January 2018 to May 2019 were chosen as the research objective. No patients showed the symptoms of sour regurgitation, hiccup, heartburn, emesis, etc., the medical history of esophageal motility disorder disease, like achalasia and gastroesophageal reflux disease and relevant medication history. This research has been approved by the Ethics Committee of the Affiliated Hospital of Hebei University, and all patients have the right to informed consent and have signed it.

3 Experimental Apparatus & Reagents

3.1 Experimental Apparatus

Electronic Analytical Balance	Shanghai JingKe Balance Co., Ltd.
Varipette 4810 Varipette 4810 Micro Pipette	Eppendorf-Netheler-Hinz Eppendorf-Netheler-Hinz, Germany
SC-15 Numerical Control Super Thermostatic Bath	Ningbo Tianheng Instrument Plant
pHS-3	
YSD-4G YSD-4G Pharmacological & Physiological Laboratory Multifunctional Apparatus	Huaibei Zhenghua Biologic Apparatus Facilities Co., Ltd.
Medlab Medlab Isolated Organ Detector	Nanjing Medease Science & Technology Co., Ltd.
Medlab Medlab Signal Acquisition System	Nanjing Medease Science & Technology Co., Ltd.
JZ101 JZ101 Muscle Tension Transducer	Nanjing Medease Science & Technology Co., Ltd.
Isolated Organ Perfusion Chamber	Nanjing Medease Science & Technology Co., Ltd.

3.2 Experimental Reagent

HCI	Tianjin Tianyi Chemical Reagents Factory
KCl	Tianjin Tianyi Chemical Reagents Factory
Glucose	Tianjin Tianyi Chemical Reagents Factory
NaCl	Tianjin Tianyi Chemical Reagents Factory
CaCl2	Tianjin Tianyi Chemical Reagents Factory
NaHCO3	Tianjin Tianyi Chemical Reagents Factory
MgSO4	Tianjin Tianyi Chemical Reagents Factory
NaH2PO4·2H2O	Tianjin Tianyi Chemical Reagents Factory
95%O2 和 5%CO2 Gas mixture of 95%O2 and 5%CO2	Shijiazhuang Xisanjiao Oxygen Manufacturing Station
TTX	BELLANCOM
NG-nitro-L-arginine (L-NOARG)	Sigma Sigma, America
Serotonin	Abcam Abcam, Britain
Methysergide maleate	Abcam Abcam, Britain
2-Methyl-5-HT	Abcam Abcam, Britain
Granisetron	Abcam Abcam, Britain
Tegaserod	Abcam Abcam, Britain
GR113808	Abcam Abcam, Britain
LP-44	Abcam Abcam, Britain
SB 269970	Abcam Abcam, Britain

4 Experimental Methods

4.1 Specimen collections & Muscle strips preparation

The specimen of the fresh gastroesophageal junction with the upper and lower parts of 3 cm taken from the operating room was placed in the Krebs at 4℃ immediately. After being washed, the specimen was fixed in the Krebs cake wax with the mucosa upward and the mixed gas of 5% CO_2 and 95% O_2 was continuously injected. A scalpel was used to dissect the specimen from the greater curvature and sharply separate the mucosa layer and the lower layer. Then, the sling fibres diagonally distributed on the greater curvature and the clasp fibres on the lesser curvature were found respectively after figuring out the locally thickened muscle layer as the lower esophageal sphincter. The sling and clasp fibres were separated along with the texture and made into muscle strips of 2 mm*10 mm. However, during the preparation, attention should be paid to carefully ensure the parallel of the long shaft of the muscle strips and the muscle bundle and prevent the muscle strips from being broken. In addition, the mucosa, its submucous membranes as well as longitudinal esophageal muscles had better be fully used. However, it should be abandoned if the specimen invaded by tumour is seen in the surgery or shown by the postoperative pathology or the resection range of the specimen is too small.

Krebs: NaCl 118.3 mmol/L, $CaCl_2$ 2.5 mmol/L, KCl 4.6 mmol/L, $MgSO_4$ 1.2 mmol/L, $NaHCO_3$ 25 mmol/L,$NaH_2PO_4 \cdot 2H_2O$ 1.0 mmol/L, Glucose 11.1 mmol/L. HCI Ph 7.40 ± 0.05.

Krebs Liquid Formula: NaCl 118.3 mmol/L, $CaCl_2$ 2.5 mmol/L, KCl 4.6 mmol/L, $MgSO_4$ 1.2 mmol/L, $NaHCO_3$ 25 mmol/L, $NaH_2PO_4 \cdot 2H_2O$ 1.0 mmol/L and Glucose 11.1mmol/L. The pH is regulated by to 7.40 ±

0.05.

4.2 Measurement of the muscle strip tension

4.2.1 Measurement of the optimal initial length of the muscle strip

The muscle strips were tied firmly with threads and vertically hang in the 10 mL Krebs liquid in the constant temperature bath at 37 ℃ and the mixed gas of 5%CO_2 and 95% O_2 was continuously injected. The lower end of the strip was fixed, while the upper end was connected to the JZ101 Muscle Tension Transducer fixed on a precisely adjustable lifting frame, and the changing situation of all muscle strip tensions was gathered by the Medlab signal collector. The lifting frame was precisely adjusted to ensure the tension of 200 mg, at which the muscle strip length was the original one L0. Then, the muscle strip at L0 was bathed warmly at 37 ℃ for 60 minutes and the Krebs liquid was updated every 20 minutes, after which the muscle strip was pulled slowly and gently once per 15 minutes at nearly 25% to twice the muscle strip length, that is, the most appropriate original length[8]. The whole experiment is conducted under the same condition, otherwise it shall be abandoned if the muscle strip tension is unstable.

4.2.2 Effects of the non-selective 5-HT receptor agonist and antagonist on the clasp and sling fibres

After the muscle strips were constant at the optimal initial length for nearly 30 minutes, the non-selective 5-HT receptor agonist 5-HT (Serotonin) was added into the 37℃ thermostatic bath in the method of accumulated concentration of 10^{-9}, 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5}, 10^{-4} and 10^{-3}mol/L. The change of the muscle strip tension after each medicine addition was carefully observed, and the medicine of next concentration would be added only when the muscle strip reached its maximum reaction and remained stable for 10 minutes. Based on this, the Concentration-Response Curve of the accumulated dose was established, and the biggest effect of medicine addition and the corresponding concentration were observed. Through observing the effects of the antagonist, the concentration of the antagonist was found same to that of the agonist that leads to the biggest effect; in addition, when the non-selective antagonist (Methysergide maleate), the concentration of the antagonist and agonist that can induce the biggest effect of the muscle strip was same.

4.2.3 Effects of the selective 5-HT receptor agonist and antagonist on the clasp and sling fibres

After the re-chosen muscle strips were constant at the optimal initial length for nearly 30 minutes, the selective 5-HT3, 5-HT4 and 5-HT7 receptor agonist (2-Methyl-5-HT, Tegaserod, and LP-44) was added into the 37℃ thermostatic bath in the method of accumulated concentration of 10^{-9}, 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5}, 10^{-4} and 10^{-3}mol/L. The change of the muscle strip tension after each medicine addition was carefully observed, and the medicine of next concentration would be added only when the muscle strip reached its maximum reaction and remained stable for 10 minutes. Based on this, the Concentration-Response Curve of the accumulated dose was established. After fully washing the muscle strips, the antagonistic effect was recorded after adding the 5-HT3, 5HT-4 and 5-HT7 receptor antagonists (Granisetron, GR113808 and SB269970) and the agonists were added after a half hour respectively. To reduce the influence of the external neurohumor on the experiment, Ng-Nitro-L-Arginine was used for the 5-HT receptor subtype with reaction on the muscle strips, which was compared with the muscle strips handled by the tetrodotoxin (TTX). (Shown in Table 1-2).

Table 1. Effect of different neurohumoral antagonists on the LES contraction induced by 2-Methyl-5-HT

Reagent	Precent of contraction(%)
control	33.5±2.8
Tetrodotoxin(1×10^{-6}M)	32.8±2.1
NG-nitro-L-arginine(3×10^{-5}M)	34.1±1.9

Table 2. Effect of different neurohumoral antagonists on the LES contraction induced by Tegaserod

Reagent	Precent of contraction(%)
control	46.4±3.4
Tetrodotoxin(1×10^{-6}M)	45.8±2.9
NG-nitro-L-arginine(3×10^{-5}M)	47.2±2.7

4.3 Statistical Analysis

Statistical software SPSS 19.0 and GraphPad Prism 6.0 were used to conduct statistical analysis. The drug induced clasp and sling fibres were expressed in the Average Contraction Percent of Muscle Strips ± Standard Error ($x \pm s$). The two-factor analysis of variance was used to analyze the comparison between the Concentration - Response Curve of the medicine. If $P<0.05$, it is considered statistically significant.

5 Results

5.1 Effects of the non-selective 5-HT receptor agonist (Serotonin) and antagonist (Methyergide maleate) on the clasp and sling fibres of the LES

The non-selective 5-HT receptor agonist (Serotonin) in the concentration of 10^{-9} and 10^{-3} mol/L can induce the contraction of the clasp and sling fibres of the LES; In the concentration of 10^{-4}mol/L, the contraction of the two muscle fibres reached the maximum. In various concentrations, no statistical significance was found in the difference in the comparison between the two muscle fibres ($P=0.67$). The non-selective 5-HT receptor antagonist (Methysergide maelate) can be used to inhibit the contraction of Serotonin on the clasp and sling fibres of the LES. The manifestation showed that no contraction happened in two fibres of the LES after adding the antagonist. (Shown in Figure 1).

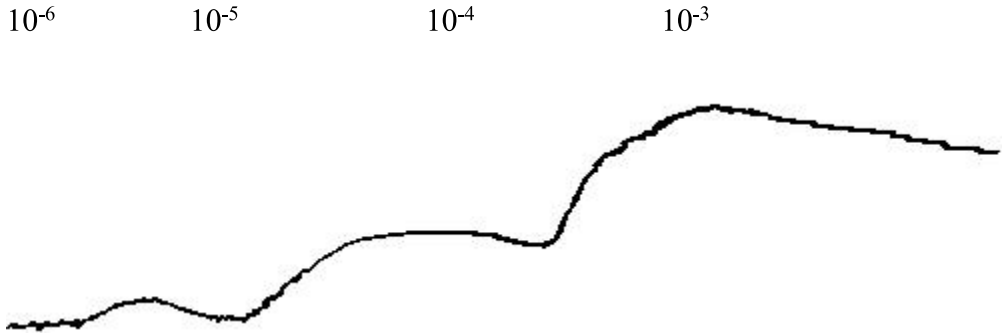

Figure 1. The non-selective 5-HT receptor agonist serotonin induced the contraction of the human LES at the concentration of 10^{-9}-10^{-4}mmol/L.

5.2 Effects of the selective 5-HT3 receptor agonist (2-Methy-5-HT) and antagonist (Granisetron) on the clasp and sling fibres of the LES

The selective 5-HT3 receptor agonist (2-Methyl-5-HT) in the concentration of 10^{-9} and 10^{-3}mol/L can induce the contraction of the clasp and sling fibres of the LES; In the concentration of 10^{-4}mol/L, the contraction of the two muscle fibres reached the maximum percentage, that is, (33.0±2.6)% for the clasp fiber and (36.0±2.3)% for the sling fiber. After the application of the selective 5-HT3 receptor antagonist (Granisetron), the contraction of muscle strips induced by the 2-Methyl-5-HT significantly decreased, with the maximum percentage of 8.4±1.5% for the clasp fiber, and (9.1±1.2)% for the sling fiber. In various concentrations, no statistical significance was found in the difference in the comparison between the two muscle fibres after the application of agonists and antagonists ($P=0.67$). Statistical difference was found in the contraction percentage of two muscle strips before and after the application of the antagonist($P<0.01$). (Shown in Figure 2-3)

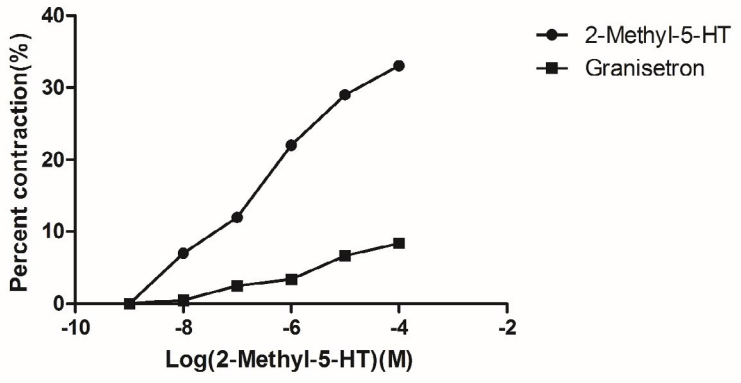

Figure 2. The contraction of clasp fibres induced by 2-Methyl-5-HT, before and after the administration of Granisetron (1×10^{-4}M) ($F=98$, $P<0.01$)

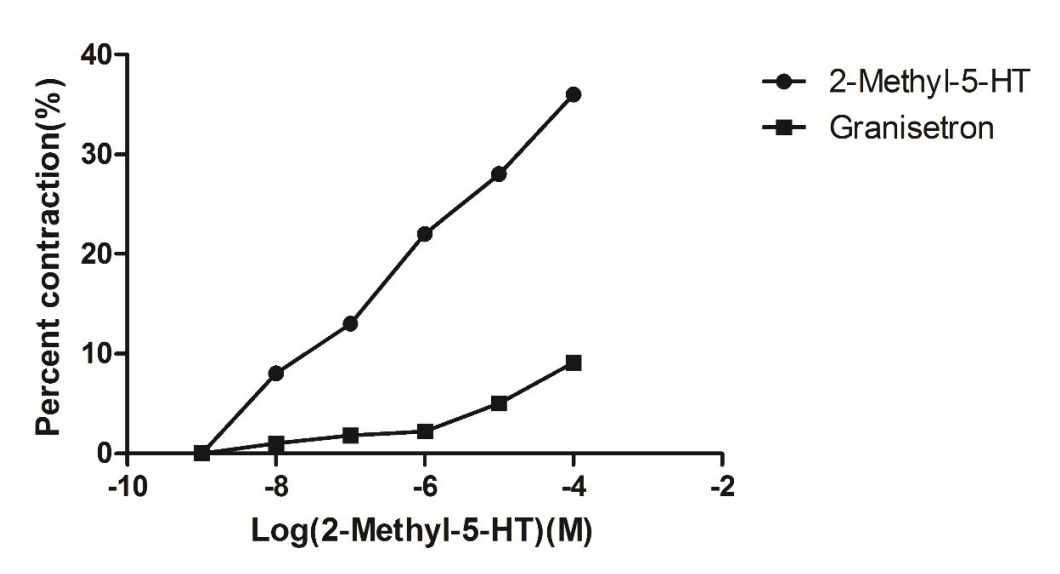

Figure 3. The contraction of sling fibres induced by 2-Methyl-5-HT, before and after the administration of Granisetron (1×10^{-4}M) (F=103, P<0.01)

5.3 Effects of the selective 5-HT4 receptor agonist Tegaserod and antagonist GR113808 on the clasp and sling fibres of the LES

The selective 5-HT4 receptor agonist (Tegaserod) in the concentration of 10^{-9} and 10^{-3}mol/L can induce the contraction of the clasp and sling fibres of the LES; In the concentration of 10^{-4}mol/L, the contraction of the two muscle fibres reached the maximum percentage, that is, (45.0±3.4)% for the clasp fiber and (47.0±2.3)% for the sling fiber. After the application of the selective 5-HT4 receptor antagonist (GR113808), the contraction of muscle strips induced by the Tegaserod significantly decreased, with the maximum percentage of (9.4±1.6)% for the clasp fiber, and (10.1±1.5)% for the sling fiber. No statistical significance was found in the difference between the contraction of the clasp and sling fibres after the application of agonists and antagonists (P=0.52). Statistical difference was found in the contraction percentage of two muscle strips before and after the application of the antagonist(P<0.01).(Shown in Figure 4-5)

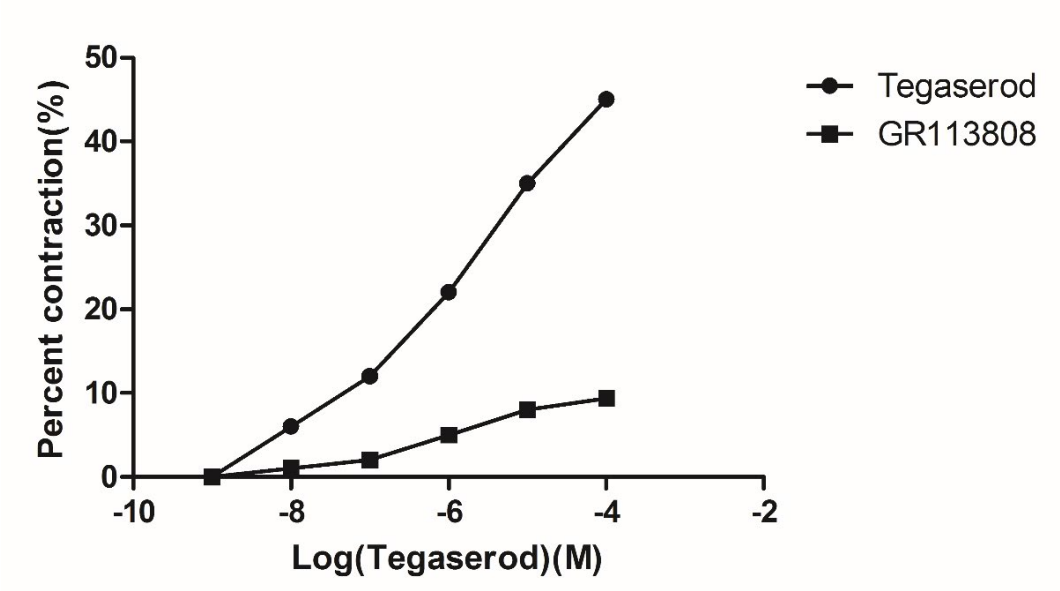

Figure 4. The contraction of clasp fibres induced by Tegaserod, before and after the administration of GR113808 (1×10^{-4}M) (F=118, P<0.01)

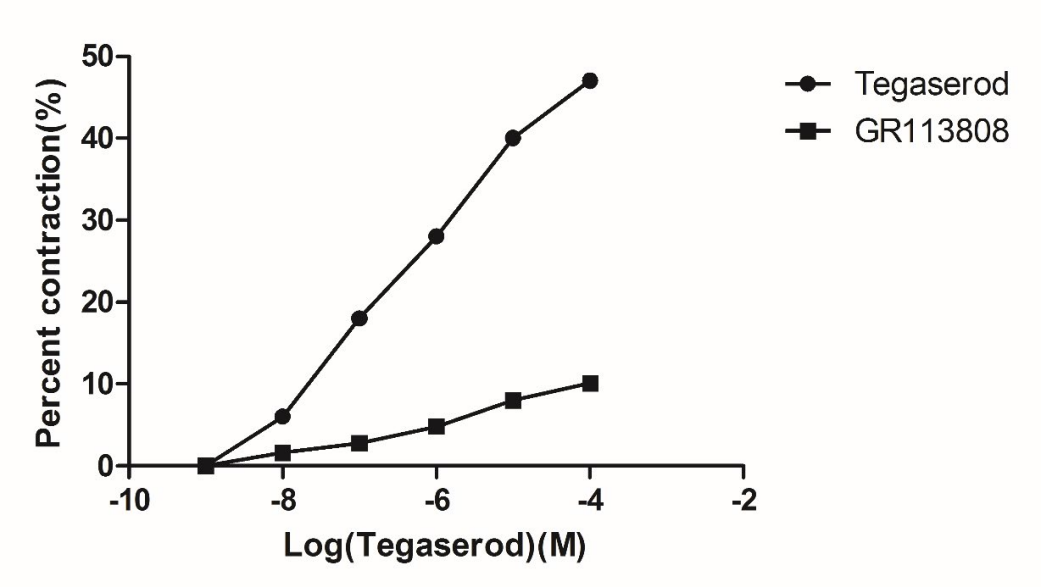

Figure 5. The contraction of sling fibres induced by Tegaserod, before and after the administration of GR113808 (1×10^{-4}M)(F=106, P<0.01)

5.4 Effects of the selective 5-HT7 receptor agonist LP-44 and antagonist SB 269970 on the clasp and sling fibres of the LES

The selective 5-HT7 receptor agonist LP-44 showed little influence on the clasp and sling fibres of the LES, and no obvious reaction of the clasp and sling fibres after the application of the 5-HT7 receptor agonist LP-44 and antagonist SB 269970 of various concentration.

6 Discussion

So far, 7 kinds of 5-HT receptors associated with human functions have been confirmed and can be further divided into 16 kinds. Due to different receptor types, only the 5-HT3 and 5-HT4 receptors that are closely related to the digestive tract functions, especially sports functions, as well as the 5-HT7 confirmed recently has been chosen as the research objective in this part of the experiment.

As a postsynaptic receptor of a ligand-gated ion channels, the 5-HT3 receptor mainly exists in the sensory neurons of the gastrointestinal tract, and can send injury information to the central nervous system[10]. The 5-HT3 receptor is mainly distributed in the myenteric mucosa and submucosal plexus. The 5-HT3 receptor mediates the neurotransmission of the myenteric neuron and the activated neurons can increase the release of the acetylcholine at the sympathetic nerve ending, and strengthen the gastrointestinal dynamics and meanwhile promote the increase of body fluid and the sense of pain[11,12]. Besides playing a significant role in central and peripheral vomiting reflex, the 5-HT3 receptor is closely related to the motor function of the digestive tract and the sensitivity of the liver. The 5-HT receptor granisetron can reduce the sensitivity of the IBS patient's colon[13]. The experiment of the guinea-pig colon shows that the faeces in the colon induced the release of the 5-HT and regulated the peristalsis of the colon by influencing the 5-HT3 and 5-HT4. Studies of Chetty *et al*[14] on the distribution and functions of the 5-HT3 receptor on the gastrointestinal tract of the murine shows that the 5-HT3 receptor widely exists in the digestive tract and is much obvious in the parts that can induce the contraction of the intestinal tract, especially the proximal colonic motor functions.

Among the 5-HT receptors currently confirmed, only the 5-HT3 receptor is ligand-gated ion channel receptor, while others are the G-protein coupling receptor (GPCR) superfamily. In the GPCR, a receptor family including 7 cross-membrane structures and coupling with protein G, the receptor couples with the excitatory protein G after activation, and leads to the activation of adenyl cyclase (AC), and further conduct the cellular signal transduction and finally plays the physiological effects. Through activating the AC, the 5-HT4 receptor adds the release of the cAMP, and activates the cAMP-dependent protein kinase A, leading to the closure of potassium channel on the cell membrane and the depolarization of the cell membrane and further opening the voltage-sensitive calcium channel, such as substance P and calcitonin gene-related peptide (CGRP). These released neurotransmitters play an important role in regulating

the digestive functions. In addition, the cAMP-induced decrease of the intracellular calcium can also influence the corresponding smooth muscles and is related to the gastrointestinal tract and vascular relaxation. Therefore, the 5-HT4 receptor is closely related to the regulation of the digestive motor functions. The effect of the 5-HT4 receptor was first verified in the guinea pig, which further verifies that the 5-HT4 receptor can enhance the activity of the AC after activation, and increase the level of the cAMP and the inflow of calcium ion. Studies showed that the effect of the 5-HT4 receptor on the gastric and intestinal smooth muscle might be excitatory and inhibitory. Due to the difference between the species and gastrointestinal positions, the effect of the 5-HT4 receptor is completely opposite. The excitatory effect is achieved via the cholinergic neurons, or mediated indirectly via the nonadrenergic noncholinergic nerve; while the inhibitory effect is to directly relax the gastric and intestinal smooth muscle. In the isolated rat esophageal experiment, Lucchelli *et al*[15] found that the selective 5-HT reuptake inhibitor could cause the relaxation reactions of the muscular layer of the esophageal mucosa and further learned that the increased release of the endogenous 5-HT further activated the effect of the 5-HT4 receptor. However, for the lower esophageal sphincter, the 5-HT4 receptor agonist can cause obvious contraction response, indicating that the 5-HT4 receptor can contract the lower esophageal sphincter and increase its pressure, which plays a significant role in maintaining the pressure of the lower esophageal sphincter. Numerous studies have verified that the 5-HT4 receptor widely existed in the muscular layers of the fundus, antrum and body of the stomach of guinea pigs, dogs and humans, and the 5-HT4 receptor agonist could activate the receptor and significantly contract the stomach[16-21]. In the recital, the 5-HT4 receptor can promote its wriggle and reflex, while the 5-Ht3 receptor cannot show an obvious effect. However, in the colon, the 5-HT4 receptor can cause the relaxation of the circular muscle[22]. The animal experiment also verified that the 5-HT could cause the relaxation of dogs' colon through the effect of the 5-HT4 on the rectum leiomyosarcoma of dogs[23]. Therefore, the 5-HT4 receptor can play different roles in various parts of digestive tract parts.

In the experiment on the digestive tract, it found that the 5-HT7 receptor could regulate the relaxation of the smooth muscle of the human colon[24] and guinea pig ileum[25]. Werner M[26] also studied and found that the 5-HT7 receptor could also inhibit the peristalsis. The further studies found that the threshold of the peristalsis increased and the intestine wall adaptation decreased[27,28] after the 5-HT7 receptor was blocked. Besides its association with motor functions, the 5-HT7 receptor was also related to the feeling of the body. Studies showed that the 5-HT7 receptor existed in the afferent nerve ending on the surface of the spinal dorsal horn, and the 5HT and 5-HT7 receptor agonist could enhance the C-fos expression on the spinal dorsal horn, which meanwhile can be inhibited by the corresponding antagonists. The spinal dorsal horn with neurons of fos, the marker of activated neurons, can mediate the transmission of the pain message. Therefore, the pain correlation between the 5-HT7 receptor and the 5-HT activation-related receptor can be inferred. As a G-protein coupling receptor, the 5-HT7 receptor can promote the formation of cyclic adenosine monophosphate, couple with sensitive adenylate cyclase (AC) of calmodulin and further increase the intracellular calcium concentration through the adenylate cyclase 1 and 8, and play the physical effect of regulation nerve conduction and muscle activity by triggering corresponding cells. Most 5-HT of the human body exist in the digestive tract, and the endogenous 5-HT plays a significant role in the digestive neurons and the 5-HT7 receptor of smooth muscle cells, and can regulate the circulatory muscle conformity. After being stimulated, the gastrointestinal mucosa can cause the release of abundant 5-HT by entero-chromaffin cells, which further activate the descending neurons with the effect on the depolarized neurons of the 5HT7 receptors after activation. Therefore, nitric oxide with strong relaxation functions can be released and then the relaxation of the smooth muscle of the digestive tract is mediated. In addition, the 5-HT7 receptor can also directly participate in the synthesis of adenosine monophosphate and cause the relaxation of the smooth muscle. The paracrine mode and neuronal stimulation exist in the process of the 5-HT works on the 5-HT7 to produce physiological effects[29-30].

The study of this part found that the nonselective 5-HT receptor agonist (Serotonin) can induce the contraction response of clasp and sling fibres, which has no significant difference; While the nonselective 5-HT receptor antagonist (Methysergide maleate) can inhibit the contraction response caused by the serotonin. Numerous studies have proved the 5-HT1 and 5-HT7 receptors after activation can induce the

relaxation effect of the smooth muscle of digestive tract, while the 5-HT2, 5-HT3 and 5-HT4 receptors after activation mainly lead to the contraction response of the smooth muscle of digestive tract. According to the experiment of the first part, we have confirmed that many 5-HT receptors existed in the lower esophageal sphincter, while the contraction of the lower esophageal sphincter caused by Serotonin indicates what induces the contraction response is the 5HT receptor.

The nonselective 5-HT receptor antagonist can inhibit the effect of Serotonin, indicating Serotonin plays its role through the 5-HT receptor. The 5-HT3 receptor agonist (2-Methyl-5-HT) and the 5-HT4 receptor agonist (Tegaserod) of different concentration (from 10^{-9} to 10^{-3}mol/L) can induce the contraction response of clasp and sling fibres, indicating these two receptors mediated the contraction response of the lower esophageal sphincter, in which the contraction response induced the 5-HT4 receptor agonist is bigger than that induced by the 5-HT3 receptor. The muscle strip contraction induced by the agonist is dependent on the concentration, that is, the bigger the concentration is, the more obvious the contraction is. In addition, the muscle strips reach the biggest contract response at the concentration of 10^{-4}mol/L, and slightly reduce at the concentration of 10^{-3}mol/L. The muscle strips might become excitatory at the concentration of 10^{-4}mol/L, which was related to the receptors that can be activated. Furthermore, the 5-HT3 receptor antagonist (Granisetron) and the 5-HT4 receptor agonist GR 113808 of the concentration of 10^{-4}mol/L can antagonize the muscle strip contraction response of the corresponding agonists, further indicting the 5-HT3 and 5-HT4 receptors mainly induce the muscle strip contraction response by the effect on their own receptors. As the recently discovered 5-Ht receptor, the 5-HT7 receptor is also related to the gastrointestinal motor functions. The relevant studies have been conducted on its effect on the relaxation of the gastrointestinal tract. However, in the experiment of this part, the 5-HT7 receptor agonist (LP-44) and antagonist (SB269970) also affected the clasp and sling fibres. In the experiment of the first part, we have already confirmed that the mRNA and protein existed in the lower esophageal sphincter. Therefore, it is speculated that no effect on the contraction functions of the lower esophageal sphincter was observed in this experiment for little expression of the 5-HT7 receptor, and low affinity of the agonist and antagonist.

To eliminate the influence of nerve and body fluid, the experiment was conducted on the 5-HT3 and 5-HT4 with activation effect on the muscle strips, which was compared with the muscle strips treated with the nitric oxide synthase inhibitor (NG-nitro-L-arginine) and tetrodotoxin (TTX), and the results indicate no statistical difference in these three kinds of muscle strips on the muscle contraction response caused by the 5-HT3 receptor agonist and 5-HT4 receptor agonist, indicating that contraction caused by the receptor is rare related to neuronal and body fluid factors.

In this part, the tension measurement technology of the isolated muscle strips was first used to study the influence of the 5-HT receptor on the contraction functions of the lower esophageal sphincter. In the experiment of this part, we have proved that the 5-HT3 and 5-HT4 receptor can the concentration-dependent contraction response in the lower esophageal sphincter, while the specific regulation mechanism of the 5-Ht receptor on the lower esophageal sphincter still need to further study. In the future, it can be identified by the study on the transduction pathway of the 5-HT receptor in the functional regulations of the lower esophageal sphincter. Moreover, the role of the 5-Ht receptor in the regulation of the lower esophageal sphincter can be further determined and new clues can be provided for the treatment of esophageal motility dysfunctions.

7 Conclusions

In the experiment of this part, the isolated muscle strip measurement technology as well as the nonselective 5-HT receptor agonists and antagonists were used to preliminarily explore the role of the 5-HT receptor in the contraction functions of the lower esophageal sphincter. And the summary is shown as follows: (1) The nonselective 5-HT receptor agonist (Serotonin) can induce the contraction response of the clasp and sling fibres of the lower esophageal sphincter. The nonselective 5-HT receptor agonist can inhibit the contraction response induced by Serotonin, indicating the 5-HT plays its role in the contraction functions of the lower esophageal sphincter through the 5-HT receptor. (2) The selective 5-HT3 receptor agonist (2-Methyl-5-HT) and the 5-HT4 receptor agonist (Tegaserod) can induce the contraction response of the lower esophageal sphincter. The selective 5-HT3 agonist (Granisetron) and the 5-HT4 receptor agonist (GR113808) can inhibit the contraction response of the agonist, indicating that the 5-HT3 and 5-HT4 receptors

may participate in the contraction regulation of the lower esophageal sphincter. (3) The selective 5-HT7 receptor agonist (LP-44) and the agonist (SB 269970) cannot produce contraction response on the lower esophageal sphincter, indicating that the 5-HT7 receptor may not get involved in the contraction regulation of the lower esophageal sphincter.

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