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The potential role of thyrotropin-releasing hormone in colonic dysmotility induced by water avoidance stress in rats

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ABSTRACT

Objective: This study sought to investigate the effect and underlying mechanism of thyrotrophin releasing hormone (TRH) on colonic contractile disorders induced by chronic water avoidance stress (WAS).

Methods: Male SD rats were exposed to daily 1-h WAS or sham WAS for 10 consecutive days. The presence of TRH in the serum and colonic mucosa were determined using enzyme immunoassay kits. Immunohistochemistry and western blotting were performed to detect the expression of TRH receptor 1 (TRH-R1). The contractions of proximal colonic smooth muscle were studied in an organ bath system. The whole-cell patch-clamp technique was used to record the currents of both L-type calcium currents ($I_{Ca,L}$) and large conductance Ca^{2+}-activated K^+ (BK_{Ca}) channels in colonic smooth muscle cells (SMCs) isolated from adult rats.

Results: Enzyme immunoassay revealed that TRH was present in both serum and colonic mucosa and that this expression increased in the WAS group. Immunohistochemistry revealed that the TRH-R1 level increased in colons devoid of mucosa and submucosa from the stressed rats as compared with the control group. TRH increased the spontaneous contractions of the longitudinal muscle and circular muscle strips in a dose-dependent manner in vitro. The effect was also confirmed in an vivo experiment, where an intraperitoneal injection of TRH in rats significantly increased fecal pellet output during a 24-h period as compared with the control group. Furthermore, intraperitoneal injection of a non-specific TRH receptor antagonist, chlordiazepoxide and a TRH-R1 antibody, partially decreased the fecal pellets of WAS rats during the 10-day stress period. Furthermore, TRH increased the peak current of L-type channels in colonic smooth muscle cells (SMCs) at a membrane potential of 0 mV, while the current of large conductance Ca^{2+}-activated K^+ (BK_{Ca}) channels was not changed following the addition of TRH.

Conclusion: TRH may be involved in the dysmotility induced by chronic stress and may have some potential clinical therapeutic use in regulating gut motility.

1. Introduction

Irritable bowel syndrome (IBS) is a clinically defined disorder characterized by symptoms and signs that give no clear indication of its pathogenesis. It is the most common functional bowel disorder worldwide and affects 7–10% of the population (Talley et al., 2015; Pigrau et al., 2016). Altered visceral perception and gut motility are the main pathophysiological features associated with IBS (Quan et al., 2015a). Many patients experience comorbid behavioral disorders, such as anxiety or depression; thus, IBS is described as a stress-sensitive chronic disorder (Moloney et al., 2015). Indeed, stress is considered as one the most significant risk factors for the development of IBS. A variety of stressor types, including physical and psychosocial stressors, play prominent roles in the development and modulation of IBS symptoms (Moloney et al., 2015). It has been shown that stress has profound effects on the gastrointestinal tract including but not limited to alterations in intestinal motility, mucosal transport, gut barrier function, and visceral perception (Moloney et al., 2015; Qin et al., 2014; Buckley et al., 2014). Moreover, chronic stress has been shown to induce adaptive changes in the release of brain-gut peptides. Previous reports have indicated that plasma hormones levels of substance P (SP), thyrotropin-releasing hormone (TRH), motilin (MTL), and cholecystokinin (CCK) in water avoidance stress (WAS) rats increased significantly and peptide YY (PYY) in WAS rats was decreased significantly (Liang et al., 2012; Lu et al., 2016; van der Schaar et al., 2013; Ter et al., 2008; Forbes and Cox, 2014). Although much progress has been made in the involvement of these brain–gut peptides in IBS, the mechanism by which they contribute to IBS pathogenesis is not fully understand.

Thyrotrophin releasing hormone (TRH) was the first hypothalamic releasing peptide to be discovered (Burgus et al., 1970; Yarbrough,

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1979). Based on its actions in regulating of thyrotrophin or thyroid stimulating hormone secretion, it was primarily considered as a physiological regulator of pituitary function (Kamath et al., 2009). Further studies identified that TRH influences the activity of vagal neurons projecting to the stomach and gastrointestinal tract, and is thought to act as a mediator of vagally stimulated gastrointestinal motility during stressful stimuli (Kamath et al., 2009; Taché et al., 2014; Bakke, 1993). Indeed, endogenous central TRH induced a rapid and long lasting contractile response in the stomach, small intestine, and colon (Kamath et al., 2009; Mitsuma et al., 1995). Data on the effects of exogenous TRH on gastrointestinal motility have shown that TRH has prokinetic effects (Kamath et al., 2009; Bond et al., 1998). The anatomical localization of TRH receptors correlates with the sites of origin of gastric vagal preganglionic neurons, and the binding of TRH to its receptor influences the activity of vagal neurons projecting into the stomach and the gastrointestinal tract (Taché et al., 2014; Lotti et al., 1986). Although these findings suggest that TRH has excitatory effects on gut motility, the interaction between stress-induced colonic hypermotility and the prokinetic effect of TRH has not been studied.

Given the role of TRH in gut motility, we investigated the possibility that TRH and its high-affinity receptor, TRH-R1, contribute to disturbances in colonic motility disorder during chronic stress. Moreover, the present study investigated the effect and mechanisms of TRH on colonic smooth muscle contractility disorders induced by chronic continuous stress.

2. Materials and methods

2.1. Chemicals

TRH and chlordiazepoxide (CDP) were purchased from Sigma Chemical Co. The TRH-R1, and GAPDH antibodies were purchased from Abcam (Abcam (Hong Kong) Ltd., Hong Kong). The TRH ELISA kit was purchased from Cusabio Biotech Co.

2.2. ELISA

Blood from the heart was collected and spun for 15 min at 3000 rpm. Serum was pipetted out and frozen for further analysis. Colonic mucosa samples were frozen on liquid nitrogen and stored at −80 °C until they were processed. The levels of TRH-like substance in the serum and mucosa were measured with the specific TRH ELISA kit using 50 μL per sample per well for the assay according to the manufacturer's instructions. Samples were analyzed in duplicate in a single assay. The final concentrations of TRH-like substance (pg/mL) in the colonic mucosa were normalized to the total protein concentration (mg/mL) and the concentration of TRH-like substance (ng/mL) in the serum was converted to uIU/mL (1 uIU/mL = 3.5*1 ng/mL).

2.3. Immunohistochemistry

The localization of TRH-R1 was examined in the proximal colon by immunohistochemistry. Formalin-fixed tissues were embedded in paraffin and cut into 4-μm-thick sections. Following antigen unmasking, the sections were incubated overnight at 4 °C with rabbit polyclonal anti-TRH-R1 antibodies (ab72179, Abcam, 1:100). They were then washed three times with phosphate-buffered saline (PBS) and incubated for 2 h at room temperature with an anti-rabbit secondary antibody in PBS/Triton and streptavidin–horseradish peroxidase. Diaminobenzidine was used as a chromogen, and hematoxylin was used for counterstaining.

2.4. Western blotting

Immediately after collection, colon samples were frozen on dry ice and stored at −80 °C until they were processed. The proximal colon devoid of mucosa and submucosa was homogenized in ice-cold RIPA lysis buffer composed of 20 mM Tris–HCl, 0.1 mM PMSF, and 5 μL/mL protease inhibitor cocktail. Sample concentration were determined by Bio-Rad protein assay (Bio-Rad Laboratories, Hercules, CA, USA). Equal amounts of proteins were loaded onto 10% sodium dodecyl sulfate (SDS) gels, subjected to SDS polyacrylamide gel electrophoresis, and electrophoretically transferred to polyvinylidene fluoride membranes (Bio-Rad, Hercules, CA, USA). The membranes were blocked for 1 h with 5% nonfat dry milk in Tris-buffered saline plus 0.1% Tween-20 (TBS-T, pH 7.6) and were incubated with rabbit anti-TRH-R1 (ab72179, Abcam, 1:1000) primary antibodies overnight at 4 °C. The membranes were then washed and incubated with a horseradish peroxidase-conjugated anti-rabbit secondary antibody (1,2000) and enhanced with a chemiluminescence substrate. The data were expressed as the band intensity ratios of the target proteins to GAPDH (ab8245, Abcam, 1:1000).

2.5. Animals

Male SD rats weighting 200–250 g (obtained from Vital River, Beijing, China) were employed in this experiment and were housed in the standard controlled environment (23 ± 2 °C; 12/12-hr light/dark cycle: dark period: 7 PM-7 AM). Equal food (pre-weight) and water were given to each rat every day. Rats were habituated to single housing in controlled environment for one week before the experiment. All empirical procedures of this study were approved by the First Affiliated Hospital of Zhengzhou University.

2.6. Water avoidance stress (WAS) protocol

The test apparatus consisted of a Plexiglass cage (45 × 25 × 25 cm) with a block (8 × 8 × 10 cm) affixed to the center of the floor. The cage was filled with warm water (25 °C) to within 1 cm of the top of the block. The rats were weighed and then placed on the block for 1 h daily for 10 consecutive days in accordance with the WAS protocol. The sham WAS (SWAS) rats were placed on the same block in a waterless container. The procedures were performed between 8:00 a.m. and 10:00 a.m. to minimize the effects of circadian rhythm.

2.7. Measurement of fecal pellet output

Baseline 24 h fecal pellet output of each rat was monitored for at least three consecutive days before WAS treatment. On the day of the experiment, each rat was exposed to WAS for one hour; the fecal pellets expelled during the period of WAS were counted. After WAS, each rat was returned to their individual standard housing cage.

2.8. Colonic muscle contraction recording in vitro

Rats were anesthetized with 10% (w/v) chloral hydrate after the last stress session. A 2-cm segment of proximal colon was removed and immediately placed in a Petri dish filled with Tyrode's buffer with the following composition: 147.0 mM NaCl, 4.0 mM KCl, 2.0 mM $CaCl_2$, 0.42 mM NaH_2PO_4, 2.0 mM Na_2HPO_4, 1.05 mM $MgCl_2$, and 5.5 mM glucose. The segment was incised along the mesenteric border and pinned in the dish with the mucosa facing up. Circular muscle (CM) or longitudinal muscle (LM) strips (3 × 10 mm, width × length) were cut in the direction of the circular or longitudinal axis after removing the mucosa and submucosa. Each fresh muscle strip was fixed in a tissue chamber containing 6 mL of Tyrode's buffer that was bubbled with a mixture of 97% O_2 and 3% CO_2 and maintained at 37 °C using a circulating water jacket. One side of the strip was pinned to a hook at the bottom of the chamber, while the other side was attached to an isometric force transducer (JZJOIH, Chengdu, China) to record muscle contractions. The muscle strip was incubated for 60 min under a resting preload of 1.0 g and washed every 30 min with Tyrode's buffer before