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• BASIC RESEARCH •

Significance of changes of gastrointestinal peptides in blood and ileum of experimental spleen deficiency rats

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Abstract

AIM: To explore the mechanism of spleen deficiency (SD) by studying the relationship of gastro-intestinal peptides level and ileal electro-mechanical activity of SD rats and cold restrain rats.

METHODS: (1) spleen deficiency (SD) model was established by feeding Houpou:Zhishi: Dahuang in the ratio of 3:3:2, 3 ml/time, for 42 days. (2) The cold restrain stress model: Animals were restrained on grille and placed in a cool water at 18 ℃ for 3 h. (3) Substance P (SP) and vasoactive intestinal peptide (VIP) levels in all layers of initial part of ileum and blood in rats were measured by radioimmunoassays (RIA) while changes of electric activity and motility in ileum of rats were recorded with electrode and strain gauge.

RESULTS: SP levels in ileum and blood of experimental SD rats were significantly higher than that of the control groups (9.89±5.65 *vs* 1.22±1.18, $P<0.005$, in ileum; 22.7±3.95 *vs* 6.60±1.47, $P<0.001$, in blood) while the VIP levels of the SD rats were significantly lower than that of the controls (3.50±2.01 *vs* 9.10±4.91, $P<0.05$, in ileum; 229.8±62.4 *vs* 560.4±151.3, $P<0.001$, in blood). As compared with the controls, the average frequency of slow electric waves (21.3±0.96 *vs* 18.2±2.28, $P<0.05$) and motility (21.5±0.58 *vs* 18±2.65, $P<0.005$) of SD rats increased obviously and the frequency of fast waves of SD rat also increased. In spontaneous recovery cases, SP levels recovered significantly (compared with the SD groups, 2.99±0.62 *vs* 9.89±5.65, $P<0.001$, in ileum; 14.4±4.22 *vs* 22.7±3.95, $P<0.001$, in blood) but did not drop to normal. After the SD rats treated with Chinese herbs (Jiawei Sijun zi Tang), SP improved (compared with SD cases, 2.20±1.25 *vs* 9.89±5.65, ($P<0.001$), in ileum; 10.7±1.88 *vs* 22.7±3.95, ($P<0.001$), in blood) and VIP in blood also improved (compared with SD rats, 485.7±229.0 *vs* 229.8±62.4, $P<0.01$) while the amplitude of motility decreased apparently (compared with the SD rats, 0.64±0.096 *vs* 0.89±0.15, $P<0.01$). The ileal SP levels of cool stress didn't change while the ileal VIP levels of cool stress became significantly lower than that of the control groups (2.87±0.87 *vs* 9.10±4.91, $P<0.01$). The blood SP levels of cool stress were significantly higher (15.60±1.83 *vs* 6.60±1.47, $P<0.001$) whereas the blood VIP levels of cool stress were significantly lower than that of the control group (153.4±70.46 *vs* 560.4±151.30, $P<0.001$).

CONCLUSION: Changes of SP and VIP levels in initial part of ileum and blood of SD rats and cool stress rats may be closely related to the gastrointestinal motility disorders presented in SD and cool stress rats. the Chinese herbs (Jiawei Sijun zi Tang) currently used have partially therapeutic effect.

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INTRODUCTION

It was well known that Spleen-Stomach theory is an important constituent of the theoretical basis of traditional Chinese Medicine. The spleen here is not synonymous with the spleen in western medicine anatomically, physiologically or pathophysiologically[1-3]. Conceptually, Spleen-Stomach theory is a comprehensive one. It mainly involves the digestive system, its vegetative nervous system, immunologic function, hemopoiesis, muscle metabolism, endocrine function, hepatic metabolic function, protein, nucleotide, energy, water and salt metabolism.

In recent years, the field of gastrointestinal hormones has expanded at a dazzling speed. The successful isolation of some gastrointestinal hormones and development of sensitive assays for their detection have led to many unexpected findings[4]. Gastrointestinal hormones as regulatory peptides appear to be major components of bodily integration and have important regulatory actions on physiological function of gastrointestinal tract[5-17]. Some studies indicated that spleen deficiency syndrome (SDS) was closely related with gastrointestinal hormones[18-21].

But up to now, the mechanisms of the relationship between gastrointestinal peptides levels and gastrointestinal functional disorder in SD still remain unclear[22-31]. We tried to explore the relationship between SDS and gastrointestinal hormones by measuring SP and VIP, and by using electrode and highly sensitive strain sensor to record alterations of ileum activity and ileum motility in SD and cool stress rats.

MATERIALS AND METHODS

Experimental animals

Healthy adult male Wistar rats (provided by Experimental Animal center, capital university of medical sciences), weighing 0.12-0.17 kg were used in this study. They were caged in an air conditioned room (23+2 ℃).

Sijunzi decoction (SJZD), composed of ginseng, Atractylodes, Poria, Glycyrrhiza, It was prepared by routine method of decocting the crude herbal medicine twice. The filtered medication was preserved in refrigerator at 4 ℃.

Fifty rats were randomly divided into five groups: (1) control group: The rats were fed standard rat chow and water ad labium. (2) experimental SD model group. by feeding Houpou: Zhishi: Dahuang (3:3:2), 3 ml/time, 42 day. (3) Spontaneous recovery group. (4) SJZD treated group. (5) cold stress group: rats were restrained on grille and placed in cool water at 18 ℃ for 3 h.

Table 3 Changes of electric-mechanical activity in ileum ($\bar{x}\pm s$)

	Main frequency (time / min)		Average frequency (time / min)		Amplitude (time / min)	
	Slow wave	Motility	Slow wave	Motility	Slow wave	Motility
Control group	17.5±2.05	18.5±1.7	18.2±2.28	18±2.65	0.30±0.26	0.43±0.31
SD group	17.4±0.79	18.0±0.69	21.3±0.96[b]	21.5±0.58[a]	0.31±0.24	0.89±0.15[b]
Spontaneous recovery group	16.8±7.8	16.8±1.3	20.5±5.5	19.1±4.85	0.25±0.103	0.77±0.65
Treated group	13.8±3.92	16.1±3.36	20.0±4.04	19.0±3.61	0.14±0.015	0.64±0.096[c]
Cool stress group	16.5±2.67	15.7±1.1	15.2±2.01	16±2.17	0.27±0.11	0.33±0.12

[a]$P<0.005$, *vs* control group; [b]$P<0.05$, *vs* control group; [c]$P<0.01$, *vs* SD group.

Measurements

Radioimmunoassay (RIA) of SP and VIP in these samples was conducted with kits purchased from Beijing HaiKerui Biological technique center. The concentrations of SP, VIP were measured with radioimmunoassay kits. Under anesthesia, the abdomen was opened and the samples were taken as follows: (1) Blood samples of 5-6 ml from the heart were collected in tubes, the plasma was immediately separated by centrifugation, then was frozen and stored at -20 ℃ until analysis. (2) The initial part of ileal tissue were removed, rinsed and weighed, then were put into a tube with boiling water. The tube was plunged into vigorous boiling water for 3 minutes, then it was cooled down and homogenized for 10 minutes. After centrifugation at 3 000 r/min for 5 minutes, the supernatant was collected and stored at -20 ℃ until assay.

All groups fasted for 18 h before operating, anesthetized by 20 % urethane, the abdomen was opened, then the sliver electrodes and strain gauge were implanted on the initial part of ileum. The changes of electric slow wave and motility of ileum were recorded. Electrode wires were passed through the abdominal muscle and fixed on the skin. All data were handled by a two-channel physiological recorder and a computer.

The cold restrain stress model: Animals were restrained on grille and placed in a cool water at 18 ℃ for 3 h.

Statistical analysis

Data were expressed as mean ± standard deviation. Experimental results were analyzed by *t* tests was determined $P<0.05$ was considered statistically significant.

RESULTS

To assess the changes of gut peptides of gastrointestinal functional disorder in SD rats and cold restraint rats, we measured the plasma levels of SP and VIP and those in the initial part of the ileum. SP levels in ileum and blood of experimental SD rats were significantly higher than those of the control groups ($P<0.005$, in ileum; $P<0.001$, in blood) while the VIP levels of the SD rats were significantly lower than those of the controls ($P<0.05$ in Ileum, $P<0.001$, in blood). In spontaneous recovery cases, SP levels recovered significantly (compared with the SD groups, $P<0.001$) and did not drop to normal. After the SD rats were treated with Chinese herbs (Jiawei Sijun Zi Tang), SP was improved (compared with SD cases, $P<0.001$) and VIP in blood was also improved (compared with SD rats, $P<0.01$. SP levels in ileum of cool stress didn' t change while the VIP levels were significantly lower than that of the controls groups ($P<0.01$). SP levels in blood of cool stress were significantly higher ($P<0.001$) while the VIP levels were significantly lower than that of the control groups ($P<0.001$), Table 1-2.

As compared with the controls, average frequency of slow electric waves ($P<0.05$) and motility ($P<0.05$) of SD rats increased obviously while the amplitude of motility decreased apparently ($P<0.05$), Table 3.

Table 1 Changes of SP and VIP in plasma ($\bar{x}\pm s$, $\mu g\cdot L^{-1}$)

Group	*n*	SP	VIP
Control group	7	6.60±1.47	560.40±151.30
SD group	8	22.7±3.95[a]	229.8±62.4[a]
Spontaneous recovery group	7	14.4±4.22[c]	332.7±119.1
Treated group	7	10.7±1.88[b]	485.7±229.0[c]
Cool stress group	7	15.60±1.83[a]	153.4±70.46[a]

[a]$P<0.001$, *vs* control group; [b]$P<0.001$, *vs* SD group; [c]$P<0.01$, *vs* SD group.

Table 2 Changes of SP and VIP in ileum ($W_B/\rho g\cdot mg^{-1}$. $\bar{x}\pm s$)

Group	*n*	SP	VIP
Control group	7	1.22±1.18	9.10±4.91
SD group	8	9.89±5.65[a]	3.50±2.01[c]
Spontaneous recovery group	7	2.99±0.62[b]	4.11±0.83
Treated group	7	2.20±1.25[b]	4.48±1.14
Cool stress group	7	0.57±0.51	2.87±0.87[c]

[a]$P<0.005$, *vs* control group; [b]$P<0.001$, *vs* SD group; [c]$P<0.01$, *vs* control group.

DISCUSSION

Spleen is one of the five solid organs, which in Traditional Chinese Medicine (TCM), does not completely match the organ designated in western medicine from the standpoint of structure, location and function. It has the functions of digesting food, absorbing and transporting nutrients to the body tissues. The spleen also serves to control the blood and to keep the blood circulating within the vessels, and takes part in the regulation of fluid metabolism[32-38]. Spleen-Stomach theory forms the basis of diagnostic approach and treatment of Spleen-Stomach disease, Spleen deficiency syndrome is a multisystem and multiorgan functional impairment, but mainly manifest as digestive tract disturbance. Experimental researches on animal model and clinical studies on spleen deficiency syndrome have yielded fruitful results in this field which lead to a better understanding of its mechanism and help open a new avenue for treatment of diseases relevant to Spleen deficiency[21,39-47]. The Spleen stomach has various physiologic functions. such as: Spleen governs transport and transformation, Spleen-stomach transforms food into nutrients which are the sources of Qi and blood. Stomach governs down-bearing function and spleen governs up-bearing which signify the motility, secretory, assimilative, absorptive and dispersing functions of upper

digestive tract, among which, gut hormones are involved[20,48-50]. Dysfunction of up-and down-bearing function of spleen-stomach can cause gastrointestinal disturbances and various spleen deficiency syndromes[1,2,19,20,51,52].

SP and VIP are both important gut Peptides, SP and VIP partly distributed in the mucosa of gastric antrum, the mucosa of the jejunum, ileum. And the central nervous system SP has a wide range of biological actions. In the intestine, VIP markedly stimulates intestinal secretion of electrolytes and hence of water. Its other actions include relaxation of intestinal smooth muscle; sphincters; dilation of peripheral blood vessels; and inhibition of gastric acid secretion.

Our previous studies included: The use of electrode and highly sensitive sensor to record alteration of gut electric activity and motility. To explore the potential role of gut peptides in spleen deficiency (SD), we studied immunoreactive Substance P, VIP, Calcitonin Gene Related Peptide (CGRP) levels in gastric antrum, duodenum and jejunual tissues in experimental SD rats by radioimmunoassys (RIA). The study suggested that motion frequency of several regions in SD rats was lower than that of control and treatment groups, respectively (P<0.05). The minimal amplitude of electric activity was also lower than that of control and treatment respectively (P<0.05). Correlation between motion frequency and its total amplitude index was different from various regions, the time of MMC was obviously less than that of the control, and the amplitude of motility was significantly higher than that of the control. The SP, VIP levels in antrum of SD rats were obviously less than that of the control, whereas, the SP and VIP levels in duodenum of SD rats were obviously higher than that of control (P<0.05), but in jejunum only SP levels increased obviously than that of the control (P<0.05). The VIP level in duodenum of SD rats was significantly less than that of treatment group (P<0.05), but VIP level of treatment group was higher than that of the control (P<0.05). As to CGRP level in antrum and small intestine, there was no obvious difference among the 3 groups.

The present study reveals changes SP and VIP in ileum of SD rats and cool stress rat. All these data imply that changes of SP and VIP levels in the antrum and the small intestine of SD rats may be closely related with the dysmotility of gastrointestinal , malabsorption and diarrhea. The Chinese herb (si junzi Tang) is capable of improving the spleen deficiency significantly and gastrointestinal electro-mechanical activity.

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