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Function and regulation of cholecystokinin octapeptide, β-endorp hin and gastrin in anorexic infantile rats treated with ErBao Granules

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Abstract

AIM To study the role of cholecystokinin octapeptide (CCK-8), β-endorphin (β-EP), and gastrin in an anorexic infantile rat model and no subsequent regulation of nose peptides by the Yunpi complex prescription ErBao Granule.

METHODS We fed infantile rats with special prepared forage. A liquid extract of ErBao Granule was administered to the rats daily for 3 weeks, CCK-8, β-EP, and gastrin concentrations in hypothalamus, gastric antrum, and plasma of the rats were measured by radioimmunoassay, and were compared with controls.

RESULTS Treatment of rats with ErBao Granule inhibited CCK-8 secretion and increased β-EP and gastrin secretion. CCK-8 concentration in hypothalamus and plasma of model control group increased significantly and correlated negatively with food intake of models, respectively. β-EP concentration in gastric antrum and plasma of model control group decreased significantly and showed a positive correlation with food intake of models, respectively. Hypothalamus concentration of β-EP was similar in models and controls. Gastrin concentration in gastric antrum of models was lower than in the blank control group, and correlated positively to food intake of models. Finally, CCK-8 concentrations in plasma of rats showed a positive correlation with plasma β-EP ($r = -0.68$, $P<0.05$).

CONCLUSION The increased plasma and hypothalamus concentration of CCK-8, decreased gastric antrum and plasma level of β-EP, and decreased gastric antrum concentration of gastrin are associated significantly with the anorexia of infantile anorexic rat models produced by special forage. ErBao Granule can reverse these changes, which may be the major mechanisms of ErBao Granule simulating feeding.

INTRODUCTION

In pediatrics it is well recognized that children may refuse to eat for long periods of time without any demonstrable organic disease, and that the origins of this anorexia are to a large extent unknown[1-3]. We investigated 300 cases of children with anorexia [4]. Among these cases (whose causes were clarified), 50.7% was caused by improper diet and feeding. The rest of them were correlated to improper diet and feeding too. Result of several studies supports this point[5-7]. In the view of traditional Chinese medicine, the principal pathogenesis of children anorexia is PiShiJianYun (dysfunction of spleen in transportation). Thus, the YunPi method (activating the spleen) is an important part of traditional Chinese medical treatment of anorexia in children and has been studied extensively by Chinese scholars[8-10]. Since 1989, ErBao Granule, a complex prescription constituted according to Yun Pi method, has been used to treat this disease and has been observed to be effective[11]. Many children who suffered from anorexia exhibited a normal appetite 2 weeks after ErBao Granule was administered. Further study for the mechanism of ErBao Granule stimulating feeding suggested that it may increase digestive enzyme secretion, and improve both intestinal peristalsis and intestinal absorption[11]. Other similar studies have reported the same result[12,13]. However, we did not think that the action of ErBao Granule on digestive function was the major mechanism of stimulating appetite, because children with anorexia showed no significant digestive

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disturbances and malnutrition as compared with healthy children.

The regulation of appetite is a complex process that consists of both central and peripheral mechanisms and involves the integration of neurotransmitters, neuropeptides, hormones and metabolic signals[14-17]. Recently, a group of peptides, distributed in the brain and gut, have been discovered to be involved in appetite control mechanism[18]. Some brain-gut peptides stimulate eating, whereas others inhibit it. Inhibitors include cholecystokinin (CCK) and somatostatin, while enhancers include β-endorphin (β-EP) and neuropeptide Y. The secretion and release of some peptides are affected by food composition, and these peptides modulate appetite as a positive feedback or negative feedback factor at the same time[19]. We suggested the loss of appetite in children with anorexia might be related to an imbalance between the brain-gut peptides[20], because the main reason of anorexia for children is inadequate diet:more protein and fat, less mineral substance and vitamins[21,22].

To study the role of CCK, β-EP and gastrin, and their potential relationship with ErBao Granule, we used an anosexic infantile rat model[23]. ErBao Granule was used to treat the rats, and CCK-8, β-EP and gastrin concentration in hypothalamus and periphery of model rats and controls were measured.

MATERIALS AND METHODS

Animals

Forty-eight Sprague-Dawley rats (24 males, 24 females, aged 35 d-40 d,60 g ± 10 g, derived from Laboratory Animal Center of our university) were randomly divided into four groups of 12: a blank control group, a model control group, an ErBao low dose groups and an ErBao high dose group. Each rat was housed individually in a regulated environment (22℃ ± 2℃, 55% ± 10% humidity and 12 h light-dark cycle).

To establish the rat model with anorexia and treat models using ErBao Granule

The anorexic rat model was established by feeding infantile rats with forage we prepared ourselves. The model forage was a compound of ingredients including milk powder, dried minced fish , sugar corn powder, and soy bean powder. We mixed the ingredients together in 1: 1: 1: 1: 2: 2: 1.6, they were shaped into cakes, each weighing about 20 g. Dried by airing, the cakes were stored at 4℃. The major composition of model forage were measured and compared with common forage. Rats in blank control group were fed with common forage and in other three groups were fed with the model forage. All rats were allowed to drink and eat at will. Fresh food was provided each day at 09:00 and each rat's food intake was measured at the same time. ErBao Granule liquid extract (offered by Vegetable Drug Research Center of Nanjing University of Traditional Chinese Medicine) was administered (ig) for twenty days, beginning on the eighth day of the experiment. ErBao liquid extract 18.8 g/kg was given to the low dose group, and 37.6 g/kg to the high dose group. Equal amounts of saline was administered in blank control group and model control group. On the 29th day of the experiment, all animals were killed after an overnight fast.

Preparation of samples and RIA of three brain-gut peptides

Blood was obtained from the orbital artery and collected into chilled plastic tubes containing 1% heparin (10 μL/mL blood sample), miscing bene immediately and then placed on ice. Samples were centrifuged at 4℃ for 3 h. Supernatant fluid was obtained and frozen at -70℃ untilanalysis. The rats were decapitated. The brain and stomach were boiled in saline for 5 min. The hypothalamus was then separated from brain, and the gastric antrum mucosa was scraped. The hypothalamus was homogenized with glacial acetic acid (1 mol/L, 1 mL). The homogenate was incubated at room temperature for 100 min. We added NaOH (1 mol/L, 1 mL) and the suspensions were centrifuged at 4℃. Supernatant fluid was obtained and frozen at -70℃ until analysis. Levels of CCK-8, β-EP and gastrin in plasma and supernatant of animals were measured using commercial radioimmunoassay. The radioimmunoassay kits for CCK-8 and β-EP were provided by the Department of Neurobiology of the 2nd Military Medical University, and the gastrin immunoassay kits, were provided by the Institute of Atomic Energy Science of China. The standard curve for CCK-8 was r = 0.998, for β-EP, r = 0.999, for gastrin, r = 0.999, all fitted the quality control criteria.

Statistics

Data are presented as the means ± SE from 12 rats per group. Statistical analysis was performed using a two-tailed Student's t test, and correlations were examined using linear correlation. $P<0.05$ was considered significant.

RESULTS

Average daily food intake of rats in four groups

On day one, we observed no significant difference in daily food intake between rats in each group. On day seven, the daily food intake of animals in model control group, low dose group and higher dose group was significantly lower than that of the blank control group. The lower food intake of rats was maintained until the end of week 4 in model control group, whereas in low dose group and higher dose group, the daily food intake of rats increased significantly at the end of week 3 (Figure 1). The results indicate that the anorexia model was established successfully and ErBao Granule was effective to the model rats[23].

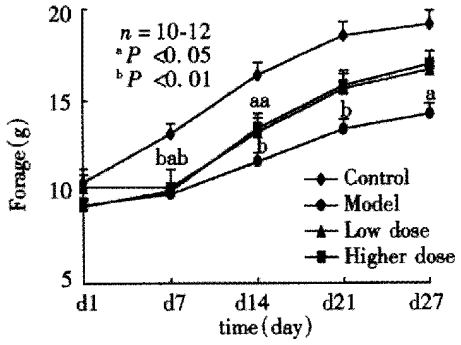

Figure 1 Daily food intake of rats in four groups. Rats of blank control group were fed with common forage. Rats of other three groups were fed with model forage. ErBao and saline were administered (ig) from the d 8 to d 28. Data are presented as the meanÀSE form 12 rats.
[a]Statistically significant difference from control, $P<0.05$, [b]$P<0.01$. At d 7, food intake of model control group, low dose group, and high dose group all was significantly lower than blank control group ($t = 3.76$, $P<0.01$; $t = 2.58$,$P<0.05$; $t = 2.83$, $P<0.01$, respectively). At d 14 food intake of the model control group, low dose group, and high dose group was still lower than blank control group ($t = 4.76$, $P<0.01$; $t = 2.53$, $P<0.05$; $t = 2.38$, $P<0.05$, respectively). At d 21, only model control group was lower than blank control group ($t = 4.71$, $P<0.01$). At d 27, still only model control group was lower than blank control group ($t = 2.33$, $P<0.05$).

Comparison of major composition between common forage and model forage

Each composition of forages was determined from parallel samples. The data were presented as the mean value. As compared with common forage, model forage showed higher concentration of coarse protein and fat (Table 1), lower concentrations of mineral elements (Table 2), and lower concentrations of vitamins (Table 3). Levels of Ca, Fe and Vitamin D were only 1/8, 1/10 and 1/7 of common forage, respectively. The results were similar to dietary survey from children with anorexia[22].

Table 1 Essential component of nutrient in two kinds of forage

Forage	Coarse protein (%)	Coarse fat (%)	Total carbohydrate (g/kg)
Model forage	18.03	39.74	389.43
Normal forage	16.12	14.99	501.05

Table 2 Content of mineral elements in two kinds of forage

Forage	Ca (%)	P (%)	Zn (mg/kg)	Cu (mg/kg)	Fe (mg/kg)
Model forage	0.17	0.08	22.7	7.24	42.39
Normal forage	1.36	0.21	40.46	9.64	428.20

Table 3 Content of vitamin in two kinds of forage (IU/g)

Forage	CaVit A	Vit D_3	Vit B_1	Vit B_2	Vit B_6
Model forage	11.1	2.3	5.3	3.3	4.7
Normal forage	21.5	17	7.3	14.3	19.9

CCK-8 level in four groups

CCK-8 concentration was significantly increased in hypothalamus (122.57 pmol/g ± 31.79 pmol/g) and in plasma (506.88 ng/L ± 113.32 ng/L) of model group compared with that of blank control group (89.15 pmol/g ± 17.94 pmol/g and 253.75 ng/L ± 95.09 ng/L). In high dose group, CCK-8 level dropped both in hypothalamus (95.55 pmol/g ± 20.68 pmol/g) and in plasma (322.14 ng/L ± 66.36 ng/L). In low dose group, it dropped to 100.00 pmol/g ± 18.83 pmol/g and 282.80 ng/L ± 75.63 ng/L, respectively. No significantly difference was observed between high or low dose group and blank control group (Figure 2). We found a negative correlation between food intake and CCK-8 concentrations of model control group in hypothalamus ($r = -0.67$, $P<0.05$) and in plasma ($r = -0.62$, $P<0.05$), and the hypothalamus level of CCK-8 was correlated positively to plasma level of CCK-8 in models ($r = 0.68$, $P<0.05$) (Figure 3).

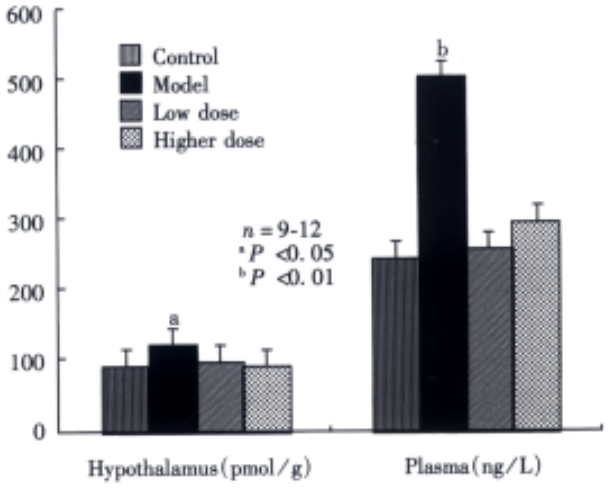

Figure 2 CCK-8 level in the four groups. Data are presented as the mean ± SE from 12 rats. [a]Statistically significant difference from control, $t = 2.55$, $P<0.05$; [b]$t = 5.81$, $P<0.01$.

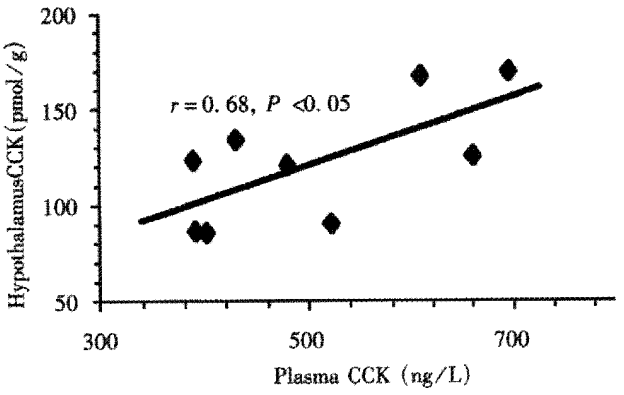

Figure 3 Correlation between hypothalamus CCK-8 and plasma CCK-8 level in model control group.

β-EP level in four groups

β-EP level in gastric antrum (1.45 μg/g ± 0.60 μg/g) and plasma (6.26 ng/L ± 1.73 ng/L) of models were significantly lower than those in the blank control group (6.28 μg/g ± 1.43 μg/g and 10.25 ng/L ± 4.86 ng/L), but hypothalamus concentration of β-EP showed no difference in rats of model control group (23.01 ng/g ± 8.01 ng/g) as compared to blank control group (24.83 ng/g ± 6.67 ng/g). In low dose group, β-EP levels in hypothalamus (28.05 ng/g ± 7.85 ng/g) and in gastric antrum (1.38 μg/g ± 0.32 μg/g) showed no obvious changes compared with model control group. Plasma level of β-EP (8.82 ng/L ± 2.86 ng/L) rose to common level. However, in high dose group, β-EP levels in hypothalamus (41.56 ng/g ± 8.74 ng/g) and gastric antrum (16.49 μg/g ± 4.07 μg/g) showed significant increases compared with those in other three groups, and plasma concentration of β-EP (8.43 ng/L ± 1.59 ng/L) raised to normal level (Figure 4). Food intake in model group correlated positively with β-EP in gastric antrum (r = 0.73, P<0.01) and in plasma (r = 0.58, P<0.05), but not in hypothalamus. We also found a negative correlation between CCK-8 and β-EP in plasma of model control group (Figure 5).

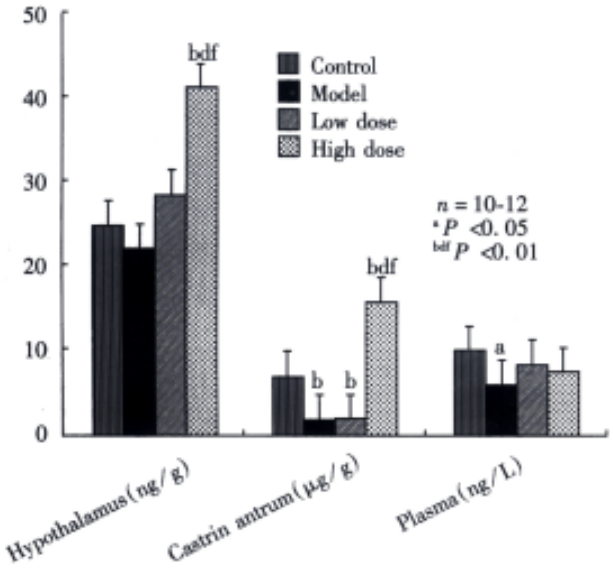

Figure 4 β-EP level between groups. Data are presented as the mean ± SE from 9-10 rats. [a]Statistically significant difference from blank control, [b]P<0.05, [d]P<0.01; from model control, [f]P<0.01; from low dose, P<0.01. In hypothalamus, β-EP level in the high dose group was higher than in the blank control group (t = 4.79, P<0.01), model control group (t = 4.82,P<0.01), and low dose group (t = 3.55, P<0.01). In gastric antrum, β-EP levels of model control group and low dose group were lower than in the blank control group (t = 9.05, P <0.01; t = 10.79, P<0.01), but β-EP level of high dose group increased more significantly than blank control group (t = 7.75, P<0.01), model control group (t = 11.34, P<0.01), and low dose group (t = 12.76, P<0.01). In plasma, β-EP level of model control group was lower than in the blank control group (t = 1.79, P<0.05).

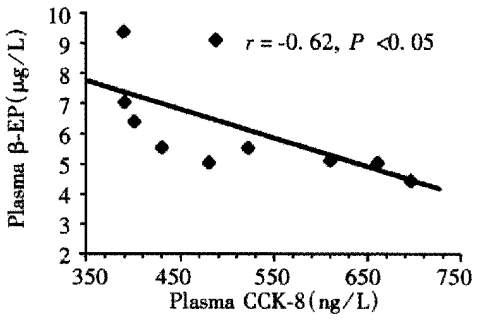

Figure 5 Correlation between plasma CCK-8 and plasma β-EP level in model control group.

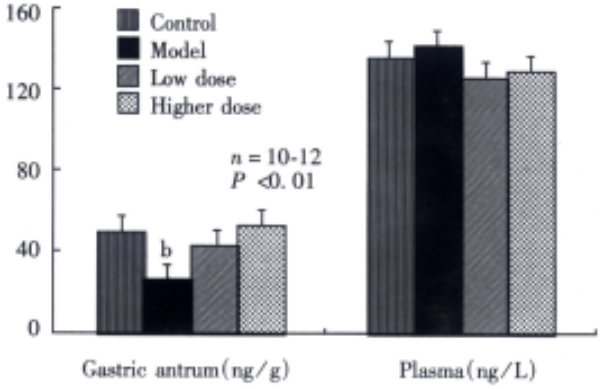

Figure 6 Gastrin level in four groups. Data are presented as the mean ± SE from 10-12 rats.[b]Statistically significant difference from blank control, t = 2.53, P<0.01.

Gastric level in four groups

Gastrin concentration in gastric antrum of model control group (27.04 ng/g ± 13.28 ng/g) was significantly lower than that of blank control group (42.78 ng/g ± 13.43 ng/g). In both the low and high dose groups, gastrin levels in gastric antrum increased to 42.78 ng/g ± 13.43 ng/g and 52.27 ng/g ± 12.37 ng/g, respectively. This increase was similar to that observed in the blank control group. Plasma concentration of gastrin remained constant in model control group, low dose group and higher dose group as compared to blank control group (Figure 6). Food intake of model control group showed a positive correlation with gastrin in gastric antrum of model group (r = 0.70, P<0.05), but not in gastrin level in plasma.

DISCUSSION

We observed an abnormal secretion status of CCK-8, β-EP and gastrin in hypothalamus and periphery of infantile rat model with anorexia produced by feeding our specially prepared forage. However, the secretion was regulated by ErBao Granule. CCK-8 is one of the most widely distributed peptides in the brain and gut. There is now strong evidence that endogenous CCK-8 is important in producing satiety in a variety of species[24,25], and it has been demonstrated to be a powerful suppressor of food

intake as compared with other peptides[26]. It has been reported that CCK-8 concentration increased in hypothalamus and gastric antrum of rat model with Spleen Deficiency[27]. No previous study has examined CCK-8 concentration in children with anorexia. However, some researchers have suggested that CCK-8 might play a role in the pathologic inhibition of food intake in idiopathic senile anorexia and anorexia nervosa [28,29]. In the present study, the model forage resulted in an increased endogenous CCK-8 secretion and release in brain and peripheral tissue of rats. Considering the negative correlation between food intake and elevated CCK-8 levels, we hypothesize that CCK-8 may be responsible, at least in part, for anorexia in children.

It remains unclear whether endogenous CCK is acting peripherally or centrally to produce satiety[30, 31]. Some studies have described that circulating CCK-8 suppressed food intake by inhibiting gastric emptying[32]. However, other investigations researches conclude that the satiety effect of endogenous CCK might not be mediated by circulating CCK[33,34]. Previous studies suggested that the satiety effect of CCK is primarily mediated by the type A receptor which is predominantly located in the periphery[35]. However, recent histochemical studies discovered that the A-type receptors are apparently present in the CNS to a greater extent than previously described[24], which indicated that CCK produces satiety by two modes:peripheral action and central action. Our study found food intake was correlated negatively with elevated CCK-8 concentration not only in plasma, but in hypothalamus of models as well, and that the hypothalamus concentration of CCK-8 was positively correlated with plasma CCK-8 level. These results agree with two modes by which CCK-8 act to inhibit food intake:one is peripheral endocrine effect, the other is a central neurocrine effect. ErBao Granule inhibited CCK-8 secretion and release both in the hypothalamus and intestines of models. We suggest that ErBao Granule might stimulate feeding by both of the two modes described above[36].

β-EP is an endogenous opiod peptide. Unlike CCK-8, β-EP can stimulate feeding significantly[37,38]. Although it has been reported that central opioids produce positive reinforcing effects, whereas peripheral opioids produce opposite effects acting as anorectic factors[39-41], a great deal of studies reported that β-EP levels in both CSF tissue and serum were reduced in anorexia of aging[42]. This suggests that β-EP concentrations in both of central and periphery play an important role in pathologic reduction of feeding. In our study, the food intake of models were positively correlated with decreased β-EP concentration in gastric antrum and plasma. We suggest that decreased peripheral concentration of β-EP might be one of the factors inhibiting feeding in models.

In the present study, ErBao Granule stimulated peripheral β-EP secretion and release. This inecreased stimulation was dose dependent, and inecreased from low dose group to the high dose group. This result indicates that the regulation of ErBao Granule on β-EP concentration in tissues was associated with dose. In addition, ErBao Granule significantly increased β-EP secretion in hypothalamus of high dose group, although there was no predominant change in hypothalamus of models, presumably because of a compensatory action in attempting to overcome anorexia. In addition, we also found a negative correlation between CCK-8 and β-EP in plasma of model group, which agree with the premise that there exists antagonism between CCK-8 and opioid system in the control of feeding behavior[43,44].

Gastrin is one of major hormones released by gastric mucosa after eating food. It has been demonstrated that gastrin is closely related to secretion and motility of the digestive tract[45,46]. Previous studies have reported abnormal high/low secretion of gastrin in patients with Spleen Deficiency syndrome and Liver-Qi Stagnation syndrome related to digestive functional disturbance[47-49]. Yet, the evidence for the role of gastrin in the control of food intake is lacking. The decreased gastrin level in serum of patients with anorexia nervosa suggested that the decrease of gastrin concentration might be one of the factors in pathologic inhibition of food intake[50]. In our study, gastrin concentration decreased in gastric antrum of models compared with controls, but not in plasma, and there was a positive correlation between higher/lower levels of gastrin and food intake in models. Therefore, we suggest that gastrin in gastric antrum might play a role in appetite control mechanisms. Furthmore, ErBao Granule may increase feeding of models by modulating gastrin secretion in gastric antrum,either directly or indirectly[51].

In summary, our results indicate that increased CCK-8 concentrations in plasma and hypothalamus, decreased β-EP level in gastric antrum and plasma, and decreased gastrin concentration in gastric antrum are associated significantly with the anorexia in rat models. The Yunpi complex prescription can regulate these changes which may be the major mechanisms of simulating feeding.

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