Cholecystokinin octapeptide (CCK-8) concentration in plasma is not affected in functional abdominal pain in children

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Abstract

Purpose: Cholecystokinin regulates gut motility and visceral sensation. The aim of the study was to determine the diagnostic value of plasma cholecystokinin octapeptide (CCK-8) concentration in children with functional abdominal pain (FAP).

Material and methods: Fifty-two children (33 girls and 19 boys) aged 6-17 years with chronic abdominal pain were included in this study. On the basis of clinical data, results of endoscopy and Criteria for Functional Disorders the patients were divided into three groups: group 1 – functional dyspepsia (FD), group 2 – irritable bowel syndrome (IBS), group 3 – non-specific FAP. The control group consisted of children without abdominal pain in anamnesis. CCK-8 concentrations in plasma were measured with radio immunoassay technique, after plasma extraction. In study protocol we analysed CCK-8 levels in fasting state and 15, 30, 60 minutes after a standard test meal.

Results: In the fasting state plasma levels of CCK-8 were similar in each group and in controls. In the IBS patients CCK-8 levels were not increased after meal. In groups 1, 3 and controls postprandial levels were higher when compared to fasting state ($p<0.05$). Area under curve of CCK-8 plasma concentration was the lowest in group 2, but not significant compared to controls and other groups. No correlation was found between main symptoms of FD and IBS and CCK-8 concentration in plasma.

Conclusions: We conclude that gut dysmotility and symptoms of functional abdominal pain in children are not concerned with alteration of plasma CCK-8 levels before and after meal.

Key words: cholecystokinin (CCK-8), functional abdominal pain.

Abbreviations: CCK-8 – cholecystokinin octapeptide, FAP – functional abdominal pain, FD – functional dyspepsia, IBS – irritable bowel syndrome, Δ AUC – Area Under Curve, IBD – inflammatory bowel diseases.

Introduction

The pathophysiology of functional abdominal pain (FAP) in children is complex. It has been revealed that motility disorders and altered visceral sensation are associated with FAP. Motor activity and visceral sensation are thought to be under control by neural mechanisms and regulatory peptides. Cholecystokinin (CCK) is one of the peptides that acts as a hormone when is released from endocrine cells in mucosa of the upper small intestine. CCK as a neurotransmitter is found in nerve fibers in myenteric and submucosal ganglia, and in smooth muscle [1]. Biological role of CCK is related not only to the stimulation of exocrine pancreatic secretion and gall bladder contraction, but also to motility of the alimentary tract. It has been shown previously that CCK inhibited both gastric acid secretion and gastric emptying [2]. This hormone has been known to relax the lower oesophageal sphincter and to stimulate colon motility by gastro-colonic reflex [3]. In experimental study, CCK exhibited potent gastroprotective activity. That protective effect of CCK-8 was accompanied by elevated plasma leptin levels and was involved in the activation of CCK-A receptor localized on vagal sensory fibers [4]. The feeling of satiety was also caused by CCK release and increased CCK activity and its satiating effect were described [5]. CCK might play a key role in gut function control. We hypothesized that disorders of regulatory mechanisms asso-

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Table 1. **Clinical features of study groups with FAP**

Study group	Diagnosis	Number of patients	Mean age (mean±SD)	Sex (female/male)	Main symptoms (% of group)
1	FD	18	11.7±2.8	12/6	upper abdominal pain (100%) headache (78%) nausea (67%) vomiting (33%)
2	IBS	8	14.1±2.5	7/1	abdominal pain (100%) distension (50%) diarrhea (37.5%) constipation (62.5%) nausea (25%)
3	nFAP	26	12.2±2.6	14/12	abdominal pain (100%) headache (77%) nausea (61.5%)
4	controls	16	11.1±3.5	11/5	-

FD – functional dyspepsia; IBS – irritable bowel syndrome; nFAP – nonspecific functional abdominal pain

ciated with gut hormone dysfunction might play a role in FAP. CCK circulates in plasma in different molecular forms (CCK-8, CCK-22, CCK-33, CCK-39, CCK-58 etc). CCK octapeptide is used as a standard in CCK assay.

The aim of the study was to determine the diagnostic value of plasma CCK-8 concentration in children with FAP. It was analysed whether fasting or postprandial CCK-8 levels in plasma could be changed in functional disturbances of the gut.

Material and methods

Fifty-two children (33 girls and 19 boys) aged 6-17.1 years with chronic abdominal pain lasting over 3 months were included in this study. Preliminary laboratory tests and procedures necessary in differential diagnosis were carried out in all the children. We included patients fulfilled Second Rome Criteria for Functional Disorders [6]. Upper endoscopies with rapid urease test were performed in children with dyspepsia and upper abdominal pain; gastritis was excluded in study group. In children with suspected irritable bowel syndrome (IBS) rectoscopy and laboratory tests were done in order to differentiate inflammatory bowel diseases (IBD). On the basis of clinical data, endoscopy and Criteria for Functional Disorders the study group was divided into three groups presented in *Tab. 1*. The control group consisted of 16 children without abdominal pain in anamnesis or alteration in the laboratory tests. These children were suspected of the respiratory tract allergy. In controls, gastro-oesophageal reflux and inflammation of alimentary tract mucosa were excluded. The study protocol was approved by the Local Bioethics Committee, Medical University of Białystok. Informed consent was obtained from the parents of children who participated in the study.

Cholecystokinin octapeptide (desulfated, CCK-8) concentrations in plasma were measured with a specific radioimmunoassay technique (RIA), using a commercially available rabbit antiserum and kit Peninsula Laboratories, Belmont, CA. Blood samples were taken at 9 a.m. from cubital vein after overnight fasting. Samples were collected in a container with ice, then immediately centrifuged at 4°C (3500 cpmin) and stored at -20°C. The concentration of the hormone was measured after plasma extraction on reverse phase columns. In study protocol we analysed CCK-8 levels in fasting state (0 minute) and 15, 30, 60 minutes after a standard test meal (296 kcal), containing of 11.1 g of proteins, 14.2 g of fat and 40 g of carbohydrates (a roll, butter, ham and tea). The incremental integrated area in the form of Area Under Curve (Δ AUC) of CCK-8 plasma concentration was calculated. Statistical analysis of the data was performed using the Mann-Whitney U-test and Student t-test. Correlations were evaluated using Spearman test and Pearson coefficient. Statistical significance was set at $p<0.05$.

Results

In the fasting state plasma levels of CCK-8 were similar in each group (15.9±12.3 pg/ml in group 1, 14.4±14.9 pg/ml in group 2, 16.5±23.2 pg/ml in group 3, 18.6±19.7 pg/ml in controls). Postprandial peaks were noted at 15 and 60 minutes in groups 1 and 3, at 30 minutes in controls. In the IBS patients (group 2) CCK-8 levels were not significantly increased after meal compared with fasting state. The overall curves of CCK-8 levels are illustrated in *Fig. 1*. We found great ranges among minimum-maximum values of CCK-8 in plasma in study groups. After the standard meal the hormone concentrations were increased in groups 1, 3 and controls. In these study groups postprandial responses of CCK-8 were statistically significant when compared to fasting state at time 0 (15.5±17.2 vs 26.8±51.3 pg/ml, $p<0.05$) (*Fig. 2*).

The incremental integrated area as area under curve (Δ AUC) of CCK-8 plasma concentration was the lowest in the group 2, but the difference was not statistically significant compared to controls and other groups (*Fig. 3*). We analysed whether altered plasma concentrations of CCK-8 might be concerned with symptoms of FAP. In IBS patients the analysis of correlation between main symptoms (dominant diarrhoea

Figure 1. **CCK-8 concentration in plasma at fasting state and after meal in study group**

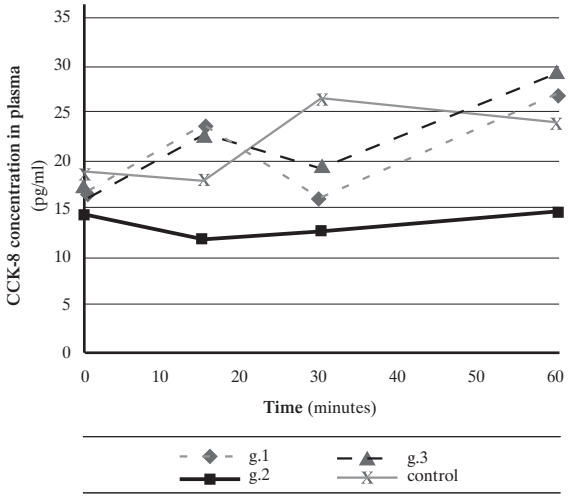


Figure 2. **Correlation between plasma levels of CCK-8 (time: 0 min vs 60 min) Pearson coefficient r=0.47**

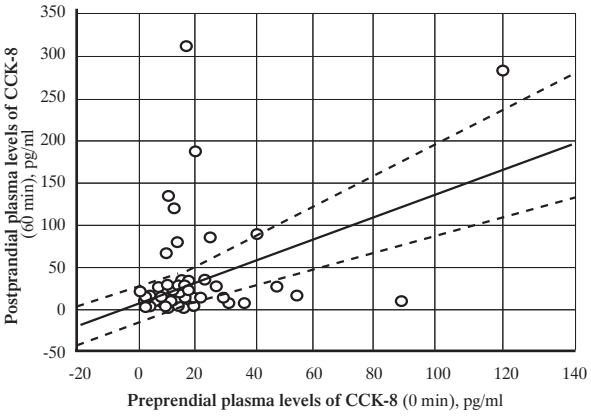
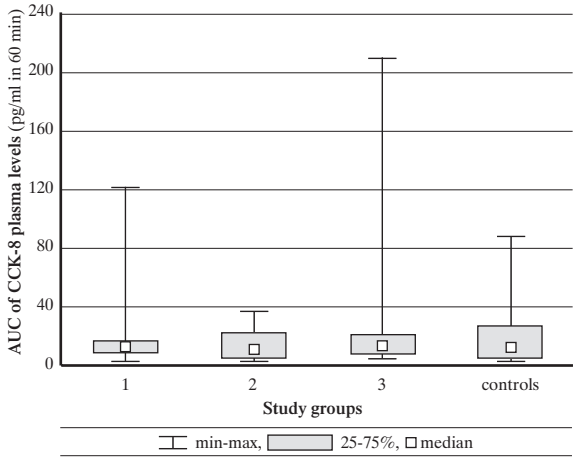


Figure 3. **Range and median value of AUC of CCK-8 in plasma**



or dominant constipation) and Δ AUC of CCK-8 concentrations proved no statistical relationship. Similarly, symptoms of dyspepsia (nausea, vomiting, upper abdominal pain) were not significantly related to CCK-8 release in Spearman test.

Discussion

It was suggested that hormonal aberrations might occur in various types of functional disorders of the gut. In our study, fasting plasma levels of CCK-8 were similar in all groups of children with functional abdominal pain and controls. Alfven and Uvnas-Moberg found significantly higher plasma CCK concentrations in children with recurrent abdominal pain, but no relationships were found between hormone levels and the occurrence of abdominal pain and other symptoms in children investigated twice [7]. The diagnostic criteria of recurrent abdominal pain have been changed and recently named functional abdominal pain (FAP) according to Second Rome Criteria [6]. Pathophysiological mechanisms of FAP were conducted in different subgroups, often in IBS-patients. Sjolund found higher fasting and peak postprandial CCK levels in IBS than in controls [8]. In our study, postprandial release was slightly impaired in IBS-patients compared with others, but not significantly. In the study of Niderau, administration of CCK stimulated motor activity in the colon [1]. However, the use of a blocker of CCK-A receptor, loxiglumide, had no effect on the meal-induced motility in the colon. These diverse results might reflect heterogeneity of IBS-group with predominant diarrhea or constipation. In another functional disorder of colon, encopresis, no significant difference was found in the measurement of CCK-patterns over time between encopretic children and control patients [9]. In the study of Peracchi et al., patients with slow-transit constipation had abnormal postprandial pattern of CCK with delayed postprandial peaks of plasma CCK (99 min in slow-transit constipation and 46 min in controls) [10]. It was previously shown that the phase of intestinal MMC at meal intake could modulate the postprandial endocrine response; plasma CCK increased earlier after intake during late phase II than after phase I [11].

The influence of CCK on motility has been evaluated in various types of gastrointestinal disorders. Elevated CCK plasma levels in patients after cholecystectomy explained the incidence of gastro-oesophageal reflux concerned with lower oesophageal sphincter relaxation [12]. In our dyspeptic patients no significant changes of fasting and postprandial CCK-8 levels were noticed compared with controls and we found no correlation between CCK-8 levels and occurrence of dyspeptic symptoms. Fried and Feinle showed that nutrient fat and distension of the stomach could modulate upper gastrointestinal sensations by postprandial release of CCK and develop dyspeptic symptoms such as nausea, bloating, pain and fullness [13]. CCK acts as mediator of gastric perception. In our study, standard meal containing 14.2 g of fat was used, which caused a postprandial increase in plasma CCK-8 levels in FD, but without influence on severity of dyspeptic symptoms. The action of CCK-8 could be one of the

causes of abdominal pain in the study performed by Chey et al. in patients with IBS [14]. It was explained by dose-dependent muscle contraction via the colon enteric nervous system mediated by CCK-8. From the other point of view CCK-antagonists might have therapeutic potential for the reflux disease, bowel motility disorders and gastroparesis [15]. These arguments explained our study protocol analysing CCK-8 plasma measurements in children with gut motility disorders.

Conclusions

The results of the study indicated no alteration of plasma CCK-8 levels in functional disorders of alimentary tract in children. Predominant symptoms in FAP were not concerned with CCK-8 release impairment. Measurement of CCK-8 plasma levels seems to have poor diagnostic value in FAP.

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