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Increased circulating cholecystokinin contributes to anorexia and anxiety behavior in mice overexpressing pancreatic polypeptide

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Abstract

We have previously reported that pancreatic polypeptide (PP) overexpressing mice display thin phenotype with delayed gastric emptying and decreased food intake. In the present study, we further examined if CCK contributes to anorexia and anxiety behavior in PP overexpressing mice. Plasma CCK levels in PP overexpressing mice and their littermates were determined by radioimmunoassay using antisera specific to sulfated CCK-8 and CCK-33. To elucidate the role of CCK in PP overexpressing mice, CCK-1 receptor antagonist (L-364,718) or saline was administered intraperitoneally and food intake was measured for 2 h. CCK-2 antagonist (L-365,260) or saline was injected intraperitoneally and the elevated plus-maze test was performed to assess anxiety. Plasma CCK levels were significantly increased in PP overexpressing mice. Administration of L-364,718 increased food intake in PP overexpressing mice compared to the saline-injected PP overexpressing group, while L-364,718 did not increase food intake in non-transgenic littermates. PP overexpressing mice exhibited anxiety in the plus-maze test. Administration of CCK-2 receptor antagonist (L-365,260) reversed the decreased percentage of entry into the open arms in PP overexpressing mice. These results indicated that elevated CCK may contribute to anorexic and anxious phenotype of PP overexpressing mice.

Keywords: Pancreatic polypeptide; Cholecystokinin; Anxiety; Anorexia; CCK receptor antagonist

1. Introduction

Cholecystokinin (CCK) and pancreatic polypeptide (PP) are gastrointestinal hormones, also known as circulating satiety factors. CCK decreases food intake influencing meal size [1] and exerts short term inhibition of food intake [2]. Both CCK and PP are secreted after meals, and modulate the digestive system. CCK enhances contraction of the gall bladder and increases pancreatic exocrine secretion [3]. On the other hand, PP reduces gall bladder contraction and decreases pancreatic exocrine secretion [4].

Peripheral and central administration of CCK stimulates PP release via vagal–cholinergic pathways [5]. However, there is no data reported as to whether PP influences CCK secretion. We have shown that PP overexpressing mice exhibited decreased food intake and body weight [6]. The concentration of plasma PP in fasted PP overexpressing mice was 4669.1 ± 279.6 pg/ml. It was obvious that high levels of plasma PP decreased food intake, since anti-PP serum in PP overexpressing mice antagonized the effect. Delayed gastric emptying contributed to the reduced food intake in PP overexpressing mice. Fasting plasma triglyceride levels were lower, while fasting blood glucose, plasma insulin, cholesterol and free fatty acid levels in PP overexpressing mice were not different from non-transgenic littermates. Recently, we reported that peripheral injection of PP increased oxygen consumption in normal mice [7], suggesting circulating PP may have a role in energy expenditure.

CCK plays an important role in anxiety while the role of PP is not yet determined. CCK-1 receptors are distributed in the periphery, while CCK-2 receptors are distributed in the brain [8]. It has been reported that CCK-1 receptors are responsible for the anorexic effect of CCK [9,10] and CCK-2 receptors influence anxiety behavior [8,11]. To examine whether or not CCK may contribute to the anorexic phenotype of PP overexpressing mice, we first determined circulating CCK levels and evaluated if CCK-1 receptor may play a role in anorexia of the animals. Anxiety behavior was also investigated in PP overexpressing mice and involvement of CCK-2 receptor was determined.

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2. Methods

2.1. Animals and measurement of plasma CCK

PP overexpressing mice were generated as described previously [6]. PP overexpressing mice and their non-transgenic littermates (12 week-old, male, $n=6$ in each group) were kept in individual cages maintained at a temperature (22 °C) and light controlled (12-hours light /12-hours dark) environment with food and water available ad libitum. The average body weights of the animals were: control 25.2±0.3 g and PP overexpressing mice 23.7±0.5 g. Blood was collected from the orbital sinus (100 μl) under anesthesia with pentobarbital (60 mg/kg). Plasma CCK was measured by radioimmunoassay using non-cross-reactive antiserum specific to the amino-terminal region of CCK-8 by a novel immunization procedure which involves first, immunization with CCK-8 peptide conjugate coupled with keyhole limpet hemocyanin (KLH), then inhibition of cross-reacting antibody formation by treatment of the animals with a potent tolerogenic conjugate of beta-alanyltetragastrin and a copolymer of D-glutamic acid and D-lysine (D-GL) [12]. All experiments were approved by our university animal care committee.

2.2. CCK receptor antagonists

CCK-1 receptor antagonist (L-364,718) and CCK-2 receptor antagonist (L-365,260) were kindly donated by Dr. Goro Katuura (Shionogi Research Laboratory, Osaka, Japan).

2.3. Feeding experiment with CCK-1 antagonist

CCK-1 receptor antagonist, L-364,718 (5 μg/kg), or saline was administered intraperitoneally to PP overexpressing mice and their littermates control in fed state. The dose was determined in our preliminary experiment. The experiment started at 10:00 and food intake was measured for 2 h after the administration. Pellets were placed on a platform in a cage and weight was recorded. Food intake was calculated by subtracting the weight of the platform with pellets after 2 h.

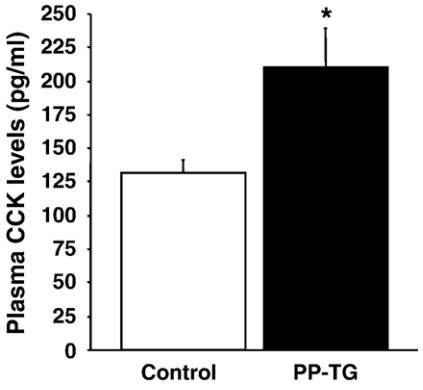

Fig. 1. Plasma CCK levels in PP overexpressing (PP-TG) mice determined by radioimmunoassay with antisera specific to CCK-8 and CCK-33. PP-TG ($n=6$); control ($n=6$). *$p<0.05$.

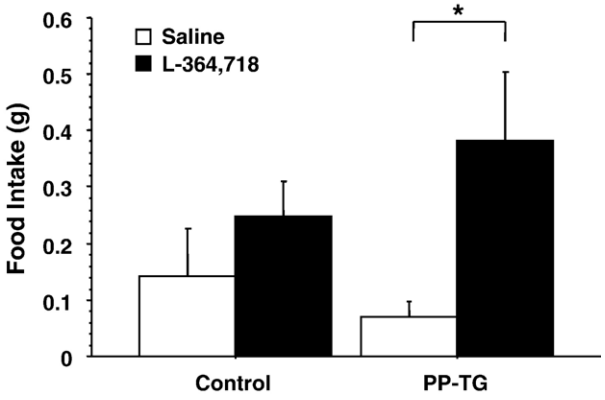

Fig. 2. The effect of CCK-A antagonist (L-364,718) on food intake. 5 μg/kg of L-364,718 was injected i.p. and food intake for 2 h was monitored. *$p<0.05$.

2.4. Anxiety behavioral test with CCK-2 antagonist

The elevated plus-maze test performed to assess anxiety in the animals was described previously [13]. Briefly, the device was placed in a dimly lit room. Two opposing arms, 50 cm above the ground, were surrounded by clear Plexiglas walls (15 cm high, closed arms), whereas the other arms were devoid of walls (open arms). The animals were placed in the center of the maze facing one of the open arms. The number of entries made into open and closed arms, in addition to the cumulative time spent there, was recorded during a 5-min session. Measurement was started immediately after the mouse entered an arm for the first time. Entry into an arm was defined as the animal placing all four paws in that arm. Saline or CCK-2 receptor antagonist (L-365,260; 10 and 100 μg/kg) was administered intraperitoneally to PP overexpressing mice and their littermates and 30 min after the injection, animals were placed into the central area facing one closed arm and allowed to explore the maze for 5 min. The time spent in the open or closed arms and the number of entries into the open or closed arms were recorded. The values were expressed as percentage of time (time spent in the open arms/time spent in open+closed arms) and percentage of entries (entries into the open arms/total (open+closed arms) entries).

2.5. Statistical analysis

Results were expressed as mean±SE and Student's t-test was used for the analysis of CCK levels. Mann–Whitney U-test was used for the analysis of feeding test. Wilcoxon signed rank test was used for the analysis of plus-maze test. Significance was set at $p<0.05$.

3. Results

3.1. Plasma CCK levels and the effect of CCK-1 antagonist on feeding behavior

Plasma CCK levels were significantly elevated in PP overexpressing mice compared to non-transgenic littermates (210.7±28.7 vs. 131.6±10.1, $p<0.01$; Fig. 1). Intraperitoneal