

論 文 要 旨

Pituitary Adenylate Cyclase-Activating Polypeptide in the Ventromedial Hypothalamus is Responsible for Food-Intake Behavior by Modulating the Expression of Agouti-Related Peptide in Mice

NGUYEN THANH TRUNG

【序論及び目的】

Although it well known that pituitary adenylate cyclase-activating polypeptide (PACAP) is most abundantly expressed in hypothalamus and contributes to hypothalamic functions, the reports in appetite regulation are discrepant. Several studies reported that exogenous administration of PACAP suppresses appetite. For example, the injection of PACAP into the lateral cerebral ventricles, ventromedial hypothalamus (VMH), paraventricular hypothalamus (PVH), central amygdala (CeA), or the bed nucleus of the stria terminalis (BNST) decreases food intake. In contrast, several reports demonstrated that daily food intake of PACAP (-/-) mice was decreased in comparison with that of PACAP (+/+) littermates. Thus, studies using (-/-) mice indicates that endogenous PACAP is orexigenic rather than anorexigenic, although its detail remains unclear. Therefore by clarifying the mechanism by which PACAP mediates its orexigenic effect, we attempted to reveal the authentic physiological role of PACAP in appetite control.

【材料及び方法】

- ◆ All experiments were carried out with male littermates or ICR mice (Japan SLC Inc., Shizuoka, Japan) that were approximately 8 - 13 weeks old.
- ◆ Feeding studies were performed in home cages with *ad lib* access to food and water. Diurnal, nocturnal, and daily food intake was assessed.
- ◆ For the fasting – refeeding paradigm, mice were fasted for 2 days and then refed at the indicated times. The body weight and food intake of mice were measured at 10 AM before and 2 days after fasting, and 4, 8 and 24 h after refeeding.
- ◆ The expression levels of agouti-related peptide (AgRP), neuropeptide Y (NPY), cocaine - and amphetamine-regulated transcript (CART), proopiomelanocortin (POMC), steroidogenic factor 1 (SF-1) and PACAP were measured by reverse transcription quantitative polymerase chain reaction (RT-qPCR) using mRNA prepared from mouse hypothalamic tissue.
- ◆ In situ hybridization and immunohistochemistry were performed using Digoxigenin (DIG)-labeled mRNA or antibodies.
- ◆ Overexpression of PACAP in the PVH and VMH. The coding region of mouse PACAP was amplified by the primer set, whose sequences were 5'- ATGACCATGTGTAGCGGAGC -3' and 5'-CTACAAGTATGCTATTCGGCGTC -3', and cloned into an pAAV-CAG::IRES-EGFP plasmid to generate pAAV-CAG::PACAP-IRES-EGFP. Adeno-associated virus (AAV) vectors (serotype 5) were produced by the AAVPro[®] Helper Free System (Takara Bio Inc., Shiga, Japan).
- ◆ The behavior test. Total travel distance and time spent in the inner ground were measured using open-

field test.

【結果】

The food consumption at 8 h after refeeding in the PACAP (-/-) mice who had fasted for 2 days was significantly lower than in the PACAP (+/+) mice. The nocturnal and daily food intake of (-/-) mice was significantly lower than those of (+/+) mice, but the diurnal food intake showed a tendency to increase. mRNA expression levels of AgRP were decreased, but those of POMC were increased in the hypothalamus of (-/-) mice 4 h after refeeding. Furthermore, intracerebroventricular administration of a PACAP receptor antagonist, PACAP₆₋₃₈ (1 nmol/ 4 μ L/ mouse), decreased food intake and body weight 1, 2, 4 h after refeeding, as well as expression levels of AgRP at 4 h after refeeding in (+/+) mice. The selective overexpression of PACAP by the infection of an adeno-associated virus in the VMH resulted in an increase in food intake and AgRP expression in the nocturnal period in addition to the increased food intake at 8 h after refeeding

【結論及び考察】

To the best of our knowledge, this study is the first to identify the mechanism underlying decreased food intake in PACAP (-/-) mice. Specifically, we showed that endogenous PACAP might work via PAC1 receptors to enhance food-intake behavior. In addition, PACAP may influence expression levels of hypothalamic neuropeptides including AgRP and POMC, known to play important roles in appetite regulation. Finally, we demonstrated that PACAP in the VMH region had an orexigenic effect during the nocturnal period and after fasting. These results suggest that food-intake behavior in mice is triggered by the increase in PACAP expression in the VMH via modulation of AgRP expression in the hypothalamus, pointing to PACAP inhibition as a potential strategy for the development of anti-obesity drugs.