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Feeding response following central administration of chicken vasoactive intestinal peptide in chicks

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ABSTRACT

Vasoactive intestinal peptide (VIP) is expressed in central nervous systems and peripheral tissues across lower and higher vertebrates and is involved in many physiological functions. One of these functions is appetite regulation; however the mechanisms mediating this response are poorly understood. Therefore, the purpose of this study was to investigate central mechanisms of VIP induction of satiety using chicks as models. Intracerebroventricular (ICV) injection of VIP (0.1 and 0.5 nmol) significantly decreased food intake under both *ad libitum* and food deprivation conditions and chicken VIP (cVIP) was more potent than mammalian VIP. The mechanisms involved with the VIP-induced anorexigenic effect were investigated by studying the involvement of the central corticotrophin-releasing hormone (CRH) systems. ICV injection of cVIP caused increased plasma corticosterone concentration and decreased diencephalic mRNA expression of CRH, CRH receptor-2 (CRH-R2) and urocortin 3 (UCN-3, which has high affinity for CRH-R2). This simultaneous decrease in the expression of ligands and their receptor, with the increase in plasma corticosterone concentration suggests that the anorexigenic effect of cVIP might be related to CRH systems. The cVIP-induced anorexigenic effect was partly attenuated by co-injection of astressin, a CRH-R2 antagonist, supporting this thesis. The present study demonstrated that VIP inhibits feeding behavior via CRH systems in the brain of chicks.

1. Introduction

Vasoactive intestinal peptide (VIP) is a member of the glucagon super family (Mutt and Said, 1974; Said and Mutt, 1970) that is distributed throughout the gastrointestinal tract and brain (Peeters et al., 1998; Sherwood et al., 2000). It has many physiological roles such as regulation of vasodilation and causes the release of several hormones of pituitary and pancreatic origin (Sherwood et al., 2000). Additionally it is associated with appetite-related responses in a range of vertebrates: intracerebroventricular (ICV) injection of VIP inhibits food intake in chicks (Tachibana et al., 2003; Tachibana et al., 2004a; Tachibana et al., 2004b), goldfish (Matsuda et al., 2005; Matsuda et al., 2006) and rats (Ghourab et al., 2001). Therefore the appetite associated effect of VIP may be conserved across vertebrates.

However, previous studies in chicks were performed with mammalian VIP (mVIP) but not chicken VIP (cVIP) (Tachibana et al., 2003; Tachibana et al., 2004a; Tachibana et al., 2004b). It has been reported that mVIP was less potent in the stimulation of cAMP production in chicken hypothalamus and cerebral cortex than chicken VIP (cVIP) (Nowak and Kuba, 2001). However, the effect of cVIP on feeding behavior has not been investigated. It is possible that cVIP might be suitable to clarify the underlying mechanism of the feeding-inhibitory effect of VIP in chickens. In addition, there are no studies which investigated the effect of peripheral injection of VIP in chicks although VIP is distributed throughout the gastrointestinal tract (Sherwood et al., 2000). Thus the anorexigenic mechanism underlying cVIP has not been clarified yet.

Corticotropin-releasing hormone (CRH) affects the release of adrenocorticotropic hormone (ACTH) and pro-opiomelanocortin (POMC)-derived peptides from the pars distalis (Zhang et al., 2001), and also regulates stress responses in the nervous, endocrine and immune systems of mammals (Berkenbosch et al., 1987; Brown et al., 1982). In parvocellular neurons of the hypothalamic paraventricular nucleus (PVN) of rats, there is extensive co-localization of VIP with CRH (Ceccatelli et al., 1989) and microinjection of VIP into the PVN stimulates ACTH and corticosterone release by activating CRH neurons (Alexander and Sander, 1995). VIP and CRH are also implicated in the regulation of feeding, locomotor and psychomotor activities in mammals (de Groote et al., 2005; Ghourab et al., 2001; Salak-Johnson et al., 2004). Similarly, VIP and CRH also regulate feeding behavior in chicks (Tachibana et al., 2003; Tachibana et al., 2004a; Tachibana et al., 2004b; Zhang

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et al., 2001). All these facts suggest that there may be some cross-talk between VIP and CRH on hormonal release and feeding regulation in chicks.

It has been reported that VIP stimulates the release of alpha-melanocyte stimulating hormone (alpha-MSH), a peptide hormone derived from the cleavage of POMC from hypothalamic explants of rats (Ghourab et al., 2001). Also, neuronal cells expressing POMC mRNA possess VIP receptors in the hypothalamic arcuate nucleus of rats (Mounien et al., 2006). These results suggest that the VIP-induced anorexia may be mediated through the modulation of POMC. However, the central mechanism of VIP-induced feeding and its relationship with CRH systems and other neuropeptides in chicks is poorly documented.

Therefore, the purpose of the study presented here was to better understand the central mechanisms mediating VIP induction of anorexia in chicks. This was accomplished by comparing the effects of cVIP and mVIP, since mVIP is less potent in the stimulation of cAMP production in chicken hypothalamus than cVIP (Nowak and Kuba, 2001). In addition, the effect of cVIP on behavioral patterns was examined. Finally, the central mechanism of VIP-induced feeding and its relationship with CRH systems and other neuropeptides in chicks were examined.

2. Materials and methods

2.1. Animals

Day-old male layer chicks (*Gallus gallus*, Julia, Ninobe Hatchery, Kagawa, Japan) were raised in a room kept at 30 °C with continuous lighting. A commercial diet (crude protein: 24%, metabolizable energy: 3050 kcal/kg, Toyohashi Feed Mills Co. Ltd., Aichi, Japan) and water were available *ad libitum* to the chicks. Chicks were transferred to their individual cages 1 day before each experiment. Before the experiment, body weight was measured and then chicks were distributed into experimental groups so that the average body weight was as uniform as possible between treatment groups. The chicks were maintained in accordance with the recommendations of the National Research Council (National Research Council, 1996).

2.2. Peptides, injections and measurement of food intake

All injections were made between 08:00 and 09:00. cVIP, mVIP and astressin were purchased from Phoenix Pharmaceuticals, Inc., Burlingame, CA, USA, Peptide Institute, Osaka, Japan and GenScript USA Inc., NJ, USA, respectively.

For the ICV injection, all peptides were dissolved in a saline solution containing 0.1% Evans Blue dye and the vehicle was used for the control treatment. ICV injections were performed according to a method reported previously (Davis et al., 1979). Briefly, the head of the chick was inserted into an acrylic box which had a hole at the top plate. The injection coordinates were 3 mm anterior to the coronal suture, 1 mm lateral from the sagittal suture, and 3 mm deep targeting the left lateral ventricle. Anatomical landmarks were determined visually and by palpation. The peptide solution was injected through the hole using a micro-syringe at a volume of 10 μl. This momentary procedure does not induce stress in neonatal chicks based on food intake and corticosterone release (Furuse et al., 1999; Saito et al., 2005). At the end of each experiment, the chicks were euthanized with an overdose of pentobarbital. The brain was then removed to confirm the accuracy of injection. Any chicks that did not have Evans Blue dye in the lateral ventricle were not used for further analyses.

For the IP injection, cVIP was dissolved in a saline solution and the vehicle was used for the control treatment. The IP injection volume was 200 μl.

2.3. Feeding behavior

Five-day-old chicks were ICV injected with 0 (control, vehicle only), 0.1 or 0.5 nmol cVIP under an *ad libitum* feeding condition. The injected dose was decided based on previous studies using mVIP (Tachibana et al., 2003; Tachibana et al., 2004a; Tachibana et al., 2004b). Then a pre-weighed feeder was given to each chick, and food intakes were measured at 30, 60 and 90 min after the injection using a digital balance at the accuracy of 1 mg.

To investigate the effect of cVIP on fasting-induced feeding, cVIP was ICV injected into 6-day-old chicks after 15 h food deprivation (the amount of time necessary for the alimentary canal to empty based on a preliminary trial). Water was freely available during the fast. Further procedures were the same as *ad libitum* feeding study.

2.4. Comparison of the anorexigenic effect of cVIP and mVIP

Seven-day-old chicks were ICV injected with saline (control), 0.5 nmol cVIP or 0.5 nmol mVIP under an *ad libitum* feeding condition. Food intakes were measured at 30, 60 and 90 min after the injection.

The fasting study was also performed using 7-day-old chicks fasted for 15 h. They received ICV injection of the same doses of cVIP and mVIP as the *ad libitum* feeding study.

2.5. Behavioral parameters

Behavioral observations were conducted for 30 min following ICV injection of cVIP. Six-day-old chicks were ICV injected with 0 (control), 0.1 or 0.5 nmol cVIP and then returned to their home cage. Their voluntary locomotion was quantified with infrared beam sensors (NS-AS01, Neuroscience Inc., Japan) and analyzed by digital data recording system software (DAS-008, Neuroscience Inc., Japan). The system counted more than 0.5 s movement as 1 unit of locomotion activity. Additionally, their behaviors were recorded with a video camera. Those were categorized into standing with locomotion, standing without locomotion and sitting, and times (sec) spent in each behavior were measured. In addition, time spent for feeding and drinking during standing period were measured. Food intake during the observation period was also measured.

2.6. Involvement of CRH systems on the anorexigenic effect of cVIP

To measure the effects of ICV injection of cVIP on plasma corticosterone concentration, 5-day-old chicks were ICV injected with 0 (control), 0.1 or 0.5 nmol cVIP under an *ad libitum* feeding condition. Food and water were then withdrawn from their cages to remove the effect induced by the difference in food intake. Blood was collected at 30 min following the injection from the jugular vein with heparin-containing micro-tubes. Blood was centrifuged (8000*g* at 4 °C for 4 min) and the plasma was collected and stored at −80 °C. Plasma corticosterone concentration was measured by enzyme-immunoassay using rabbit anti-corticosterone antibody (420E, Cosmo Bio Co., Ltd., Tokyo, Japan). The intra- and inter-assay variations were 6.7% and 5.3%, respectively.

Subsequently, the effect of ICV injection of cVIP on CRH, CRH receptor-1 (CRH-R1), CRH receptor-2 (CRH-R2) and urocortin-3 (UCN-3), a CRH family peptide that has high affinity for CRH-R2 mRNA expression in the diencephalon was investigated. Seven-day-old chicks were ICV injected with 0 (control) and 0.5 nmol cVIP under an *ad libitum* feeding condition. After the injection, food and water were removed to eliminate the effect of food intake. Thirty min later, the brain was removed and then its diencephalon was collected. Briefly, the telencephalon, cerebellum and optic tectum were removed in order from the whole brain with a surgical