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Central administration of prolactin-releasing peptide shifts the utilities of metabolic fuels from carbohydrate to lipids in chicks

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HIGHLIGHTS

- Central administration of prolactin-releasing peptide stimulates feeding behavior in chicks.
- Prolactin-releasing peptide decreases respiratory quotient.
- Plasma glucose and insulin concentrations are decreased by prolactin-releasing peptide.
- Prolactin-releasing peptide might be related to glucose and lipid metabolism in the brain of chicks.

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ABSTRACT

We have recently identified prolactin (PRL)-releasing peptides (PrRPs) and their stimulating effects on feeding behavior in chicks. To investigate further metabolic functions of PrRP, the present study was performed to clarify whether intracerebroventricular (ICV) injection of PrRP31, an active form of PrRP in chicks, affects heat production (HP), respiratory quotient (RQ) and plasma concentrations of metabolic fuels in chicks. The ICV injection of PrRP31 (94 and 375 pmol) did not affect HP but significantly lowered RQ. The change in RQ implies that PrRP31 shifted the utility of metabolic fuels in the body. This idea was confirmed by subsequent results in which ICV injection of PrRP31 significantly reduced glucose but increased non-esterified fatty acid concentrations in plasma. These shifts in blood metabolic fuels would not be through the increased plasma insulin, because the ICV injection of PrRP31 significantly decreased plasma insulin concentration. On the other hand, ICV injection of another orexigenic peptide, neuropeptide Y (NPY) also induced the insulin release and the metabolic effects were similar to those of PrRP31. Because ICV injection of PrRP31 increased NPY mRNA in the diencephalon, the NPY may mediate the metabolic functions of PrRP31. In summary, the present study suggests that central PrRP31 shifts the utilities of peripheral energy sources, which is not via hyperinsulinemia but via the diencephalon.

1. Introduction

A number of orexigenic peptides have been identified in the mammalian brain [1]. Some of these induce anabolic actions by decreasing energy expenditure in addition to stimulating food intake [38]. Neuropeptide Y (NPY) is one of the orexigenic and anabolic peptides recognized in mammals [38] since it induces hyperphagia [4], hypothermia [6], hyperinsulinemia [20] and a decline of energy expenditure [2] when injected centrally. The chronic central injection of NPY over several days also induces obesity-like hormonal and metabolic changes [39].

On the other hand, whether orexigenic peptides have anabolic actions is unclear in non-mammals including chicks. In the first place, some orthologs of mammalian orexigenic peptides, such as orexins, melanin-concentrating hormone and motilin, do not stimulate feeding behavior in chicks when administered centrally [7,8]. Intracerebroventricular (ICV) injection of agouti-related peptide shows an orexigenic effect only in layer-type (lower growth rate) chicks, but not broiler-type chicks (higher growth rate) [32]. Ghrelin apparently suppresses food intake in chicks after ICV injection [8,27], which is completely opposite to its orexigenic effects in mammals [23]. Collectively, these data suggest that the role of orexigenic peptides in the anabolic state is different between chicks and mammals. In fact, even central injection of NPY does not decrease energy expenditure in chicks, while it induces hyperphagia and hypothermia in chicks as in mammals [34].

Prolactin (PRL)-releasing peptide (PrRP) was first isolated from the hypothalamus as a specific PRL-releasing factor for mammalian

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pituitary cells [11]. C-terminal RF (arginine–phenylalanine) amide peptides (Carrasius RF amide; C-RFa), which are structurally related to mammalian PrRPs, were also identified as PrRPs in several fishes [21,22,29,30]. Further studies revealed that one of the physiological effects of these PrRP is suppression of feeding behavior: ICV injection of PrRP decreases food intake in rats and goldfish [13,17,28]. Furthermore, ICV injection of PrRP is also demonstrated to increase oxygen (O_2) consumption in rats [18]. Knockout of GPR10, a PrRP receptor, induces hyperphagia, obesity and the increase of leptin and insulin levels in the blood in mice [9]. Furthermore, streptozotocin-induced diabetic rats and fa/fa Zucker diabetic rats show low expression of PrRP mRNA in the hypothalamus and brainstem [19]. These facts suggest that PrRP is also associated with energy balance in mammals. In the brain of chicks, two types of PrRP were also isolated [35]. These are different in the number of amino acid: one consists of 20 amino acids and the other consists of 31 amino acids (PrRP31) [35]. Since this PrRP31 has induced PRL release and affected food intake in chicks, it has been identified as an active form of PrRP [35]. In chicks, however, the role of PrRP in feeding behavior is totally different from mammals and fish because ICV injection of chicken PrRP31 stimulates feeding behavior in chicks [35]. Furthermore, PrRP's anabolic actions have not yet been investigated in chicks.

In the present study, we investigated the effect of ICV injection of PrRP31 on heat production (HP), respiratory quotient (RQ) and plasma metabolites such as glucose (GLU), triacylglycerol (TG) and non-esterified fatty acid (NEFA) in chicks. The effect of PrRP31 on plasma concentration of insulin which is one of the central hormone to carbohydrate metabolisms in chicks [16,36] was also investigated. These effects were compared with those of the other orexigenic peptide, NPY, and the mode of the PrRP's metabolic action has been suggested by further analyses the effect on NPY mRNA expression in the diencephalon.

2. Materials and methods

2.1. Animals

Day-old male chicks (Single Comb White Leghorn, Julia, Murata Hatchery, Fukuoka, Japan and 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. Immediately before the experiment, body weight was measured and chicks were distributed into experimental groups so that the average body weight was as uniform as possible among the groups. The chicks were maintained in accordance with the recommendations of the National Research Council [24].

2.2. Peptides and injection

Synthetic chicken PrRP31 and bovine NPY (purchased from Peptide Institute, Osaka, Japan) were dissolved in a 0.1% Evans Blue solution prepared in a saline solution. The vehicle was used for the control treatment. ICV injections were performed according to a method reported previously [5,35]. In brief, the head of the chick was inserted into an acrylic box with a hole in 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 (10 μl) was injected through the hole using a micro-syringe. This procedure is quick and does not cause stress in neonatal chicks judging from food intake and corticosterone release [7,27]. All injections were made under an ad libitum feeding condition. 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 in which the presence of Evans Blue dye in the lateral ventricle was not verified were not used for further analyses.

For the insulin study, human insulin (Wako Pure Chemical Industries, Ltd., Osaka, Japan) was dissolved in a saline solution for intraperitoneal (IP) injection and the injected volume was 0.2 ml. The vehicle was used as the control treatment. The doses of PrRP31, NPY and insulin were determined based on previous studies in chicks [34–36].

2.3. Effect of ICV injection of PrRP31 on HP and RQ

To investigate HP and RQ, O_2 consumption and carbon dioxide (CO_2) production were assessed using an open-circuit calorimeter system (MK-5000RQ, Muromachi Kikai Co. Ltd., Tokyo, Japan) as reported previously [34]. An acrylic chamber (150 mm × 150 mm × 150 mm) with a stainless steel grid floor was used. Fresh atmospheric air was drawn in at a rate of 500 ml/min and then passed through O_2 and CO_2 detectors (MM202R, Muromachi Kikai Co., Ltd., Tokyo, Japan). The concentrations of these gases were recorded every 3 min. The analyzer was calibrated every hour using primary gas standards of high purity (Sumitomo Seika Chemicals Co. Ltd., Osaka, Japan). HP was calculated according to the following formula [26]: HP (kcal) = volume of O_2 consumed (ml/min) × 3.871 + volume of CO_2 produced (ml/min) × 1.194. The units for HP were converted from calories to joules by multiplying by 4.184 and standardizing with the body weight. RQ was calculated by dividing CO_2 production (ml/min) with O_2 consumption (ml/min).

One hour before ICV injection, each chick (3 days old) was transferred to the test chamber to allow acclimation to the experimental conditions. Thereafter, the chick was injected with 0 (control, vehicle only), 94 or 375 pmol PrRP31. The O_2 consumption and CO_2 production were determined for 1 h after the injection. During this period, no food or water was provided.

2.4. Effect of ICV injection of PrRP31 on plasma metabolite concentrations

Four-day-old chicks under an ad libitum feeding condition were ICV injected with 0 (control), 94 or 375 pmol PrRP31. After the injection, the diet and water were removed from their home cages. Blood was collected from the jugular vein 30 min after the injection. The blood was then centrifuged at 4 °C, 9000 ×*g* for 4 min and the plasma was collected and stored at −80 °C. Plasma GLU, NEFA and TG concentrations were determined by glucose oxidase, acyl-CoA synthetase-acyl-CoA oxidase and glycerol-3-phosphate oxidase-3,5-dimethoxy-N-ethyl-N-(2′-hydroxy-3′-sulfopropyl)-aniline sodium methods, respectively, using commercial kits (Wako Pure Chemical Industries, Ltd., Osaka, Japan).

2.5. Effect of IP injection of insulin on plasma metabolite concentrations

This study was performed to confirm the effect of insulin on plasma metabolite concentrations in chicks. Six-day-old chicks were IP injected with 0 (control), 8.5 or 34 nmol/kg insulin under an ad libitum feeding condition. The subsequent procedures were the same as those described in Section 2.4.

2.6. Effect of ICV injection of PrRP31 on plasma insulin concentration

To investigate the effect of PrRP31 on insulin release, plasma insulin concentrations were measured 15 and 30 min after the ICV injection of PrRP31. Five-day-old chicks under an ad libitum feeding condition were ICV injected with 0 (control), 94 or 375 pmol PrRP31, and then food and water were removed from their cages. Blood was collected from the jugular vein 15 and 30 min after the injection and the subsequent procedures were the same as those described in Section 2.4. Plasma insulin