Physiology & Behavior 125 (2014) 57–61

Contents lists available at ScienceDirect

Physiology & Behavior

journal homepage: www.elsevier.com/locate/phb

Comparison of brain urocortin-3 and corticotrophin-releasing factor for physiological responses in chicks

Madoka Ogino [a], Aki Okumura [a], Md. Sakirul Islam Khan [a], Mark A. Cline [b], Tetsuya Tachibana [a,*]

[a] Department of Agrobiological Science, Faculty of Agriculture, Ehime University, Matsuyama 790-8566, Japan
[b] Department of Animal and Poultry Sciences, Virginia Polytechnic Institute and State University, 24061 Blacksburg, VA, United States

HIGHLIGHTS

- Central injection of UCN-3 inhibits feeding and crop emptying rate in chicks.
- The injection of UCN-3 increases rectal temperature in chicks.
- These effects are similar to CRH, but UCN-3 induced behavior is different from CRH.
- Physiological roles of CRH receptor seem to be different between subtypes in chicks.

ARTICLE INFO

Article history:
Received 18 October 2013
Accepted 18 November 2013

Keywords:
Behavior
Chick
Corticotropin releasing hormone
Crop-emptying rate
Food intake
Rectal temperature
Urocortin-3

ABSTRACT

Corticotrophin-releasing hormone (CRH) plays an important role in response to stress, and exerts a physiological effect via its receptor, CRH receptor type-1 (CRH-R1) and CRH receptor type-2 (CRH-R2) with high affinity to CRH-R1 in mammals. Urocortin-3 (UCN-3), a CRH family peptide, is an endogenous ligand for CRH-R2 in mammals. The physiological roles of UCN-3 and CRH-R2 have been investigated in mammals, although their roles still need to be clarified in chicks (*Gallus gallus*). Few studies have been performed comparing the physiological responses of CRH and UCN-3 in chicks. Therefore the present study was conducted to investigate the effect of intracerebroventricular (ICV) injection of UCN-3 on food intake, rectal temperature, crop-emptying rate and behaviors in chicks, and to compare these physiological responses with the effects resulting from CRH injection. The ICV injection of 20 and 80 pmol UCN-3 decreased food intake, increased rectal temperature and decreased crop-emptying rate and the results were similar to those achieved with CRH. The injection of both UCN-3 and CRH increased spontaneous activity but the behavioral patterns were different: CRH increased the number of vocalizations while UCN-3 increased the number of jumps, wing-flaps and scratching behaviors. These results suggest that UCN-3 regulates food intake, body temperature, and gastric emptying via the CRH-R2 in the brain of chicks, and these effects were similar to those induced by CRH.

1. Introduction

Corticotropin-releasing hormone (CRH) is a neuropeptide [1] and plays an important role in regulating endocrine, autonomic, behavioral and immune responses to stress [2]. To date, two major types of CRH receptors have been found in mammals: one is CRH receptor-1 (CRH-R1) and the other is CRH receptor-2 (CRH-R2) [3,4]. CRH has 40-fold higher affinity to CRH-R1 than CRH-R2 in mammals [3], and induces anorexia [5], hyperactivity [6,7], inhibition of gastric emptying [8–10] and hyperthermia [6,7] when administered centrally.

In addition, urocortin-1 (UCN-1), urocortin-2 (UCN-2) and urocortin-3 (UCN-3) have been found as endogenous ligands for CRH receptors with distinct affinity to these receptors [3]. UCN-1 has high affinity to both receptors, while UCN-2 and UCN-3 selectively bind to CRH-R2. Based on their affinity, CRH has been considered as an endogenous ligand for CRH-R1, and UCN-2 and UCN-3 for CRH-R2 [3]. In rodents, central injections of UCNs induce anorexia [11–15], inhibition of gastric emptying [16–18], hyperthermia [19,20] as well as CRH [5–10].

The effect of CRH family peptides on the release of adrenocorticotropin hormone (ACTH) and corticosterone is different. CRH stimulates the releases of ACTH and corticosterone while UCNs have no effect [13,21]. The difference can be explained by their affinity to receptors. Thus the physiological functions of CRH family peptides are dependent on their receptors.

In chickens, CRH has been identified and its amino acid sequence is the same as rodents and human [22]. Chicken CRH and its receptors are distributed in the brain of avian [23,24]. Moreover, the physiological effects of CRH in chicks are almost similar to mammals. Intracerebroventricular (ICV) injection of CRH inhibits feeding behavior [25–28],

* Corresponding author at: Laboratory of Animal Production, Department of Agrobiological Science, Faculty of Agriculture, Ehime University, Matsuyama 790-8566, Japan. Tel./fax: +81 89 946 9820.
E-mail address: tetsu@agr.ehime-u.ac.jp (T. Tachibana).

0031-9384/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.physbeh.2013.11.006

and induces hyperactivity [29,30] and hyperthermia [30] as observed in mammals. However, UCN-1 and UCN-2 have not been identified in chickens. Only UCN-3 has been identified in chickens although its physiological roles have not been clarified. Recently we found that ICV injection of UCN-3 had no effect on plasma corticosterone concentration while its concentration was increased by the injection of CRH (unpublished data). This result is similar to mammals, suggesting that the physiological role of UCN-3 is different from CRH and the difference could be dependent on their receptors. However, the physiological role of CRH-R1 and CRH-R2 has not been investigated in chicks.

In the present study, we investigated the effect of ICV injection of UCN-3 on food intake, rectal temperature, crop-emptying rate and behavior in chicks. Furthermore, those effects were compared with CRH to clarify the role of CRH receptors in chicks.

2. Materials and methods

2.1. Animals

Day-old male layer chicks (*Gallus gallus*, Julia, Nihon Layer, Gifu, 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 prior to 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 [31].

2.2. Peptides and ICV injections

All injections were made between 0800 and 1000. Chicken CRH and human UCN-3 (92% homology with chicken) were purchased from Peptide Institute (Osaka, Japan) and Phoenix Pharmaceuticals Inc. (Burlingame, CA, USA), respectively.

All peptides were dissolved in a normal saline solution containing 0.1% Evans Blue dye and the vehicle was used for the control treatment. For UCN-3, dimethyl sulfoxide was added at 5% total volume to aid in dissolution. ICV injections were performed according to a method reported previously [32]. 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 microsyringe at a volume of 10 μl. The injection procedure is rapid and does not result in additional stress to neonatal chicks judging from food intake and corticosterone release data [33,34]. 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 show the presence of Evans Blue dye in the lateral ventricle were not used for analyses.

2.3. Food intake

Five-day-old chicks were ICV injected with 0 (control), 20 or 80 pmol CRH or UCN-3 after a 12-h food deprivation. The doses of these peptides were decided based on our previous study on food intake [30]. Then a pre-weighed feeder was given to each chick, and food intake was measured at 30, 60 and 90 min after the injection using a digital balance with an accuracy of 1 mg.

2.4. Rectal temperature

To determine rectal temperature, a 19 mm stainless sensor connected to an electronic thermometer (BAT7001H, Physitemp Instruments Inc., New Jersey, USA) was inserted in the chick rectum. Immediately before injection, the basal temperature of each chick (4 days old) was determined. Feed and water were available ad libitum prior to and during the temperature determination. Then chicks were injected with 0 (control), 20 or 80 pmol CRH or UCN-3 and food and water were removed. Rectal temperature was determined at 30 and 60 min after injection.

2.5. Crop-emptying rate

Crop-emptying was measured based on the method previously reported [35]. Seven-day-old chicks were food-deprived for 15 h to empty residual ingestion within the crop, and ICV injected with 0 (control), 20 or 80 pmol CRH or UCN-3. Immediately following injection, chicks were gavaged a feed slurry at a mass of 4.0% body weight into the crop. The feed slurry was made by mixing 40% powdered diet with 60% distilled water on a weight basis. No chicks vomited post-gavage in the present study. After gavage, chicks were returned to individual cages and feed and water were withheld. One and 2 h after injections, chicks were deeply anesthetized by inhalation of diethyl ether, after which their crops were exposed, the upper and the lower esophagus clamped and the crop excised. Total content of the crops were recovered and were dried at 55 °C for 48 h, and further air-dried for 24 h. The air-dried slurry was weighed using a digital balance with a precision of 1 mg. Based on the dry weight, the wet slurry weight was calculated. The weight of slurry which had emptied from the crop through the lower esophagus was calculated by subtracting the weight of slurry within the crop from the weight of administered slurry. Crop-emptying rate was expressed as the percentage of slurry emptying from the crop to the amount gavaged.

2.6. Behavior

Behavioral observations were carried out for 30 min following ICV injection of CRH and UCN-3. Five-day-old chicks were ICV injected with 0 (control) or 80 pmol CRH or UCN-3 and then returned to their home cage. Feed and water were removed. Voluntary activity of the chicks was quantified with infrared beam sensors (NS-AS01, Neuroscience Inc., Tokyo, Japan) and analyzed by digital data recording system software (DAS-008, Neuroscience Inc., Tokyo, Japan). The system counted movement of 0.5 s or more as 1 unit of locomotion activity. Additionally, their behaviors were recorded with a video camera, and the number of vocalization, jumping, wing-flapping, preening and scratching behaviors was counted.

2.7. Statistical analysis

Data of food intake, behavioral parameters and rectal temperature were analyzed with repeated two-way analysis of variance (ANOVA) with respect to peptide and time, and with Tukey–Kramer test (food intake) or *t*-test (behavioral parameters and rectal temperature) at each time point. Data of crop emptying were analyzed with two-way ANOVA and then with Tukey–Kramer test as a post hoc test. Data are expressed as means ± SEM and statistical significance was set at $p < 0.05$. The numbers of chicks are described in the legends of the figures.

3. Results

3.1. Food intake

ICV injection of 20 and 80 pmol CRH significantly decreased food intake of chick at all recorded time periods [$F(2,18) = 19.47$, $P < 0.05$]