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Acute injections of corticosterone, norepinephrine and epinephrine retards food passage in the crop of chicks

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ABSTRACT

The purpose of the present study was to clarify whether acute injection of stress-related hormones, corticosterone (CORT), norepinephrine (NE) and epinephrine (E) affect food passage in the crop of chicks (*Gallus gallus*). Subcutaneous (SQ) injection of CORT significantly retarded the food passage in the crop of chicks. Intraperitoneal (IP) injection of NE and E also significantly decreased the crop emptying rate. Additional experiments by using agonists of adrenergic receptors found that IP injection of phenylephrine and clonidine but not isoproterenol retarded the food passage in the crop of chicks. These results demonstrated that the effect of NE and E would be mediated by alpha-1-, alpha-2- rather than beta-adrenergic receptor. Finally, we found that injection of CORT, NE and E had no effect on the number of defecations while intracerebroventricular injection of corticotropin-releasing hormone and urocortin-3 significantly increased it. These results suggest that CORT, NE and E might affect the food passage in the upper digestive tract in chicks.

1. Introduction

Stressors induce many physiological responses which are regulated by complex pathways. These include the endocrine and autonomic nervous systems which are well studied in vertebrates, especially in mammals. In the endocrine system, corticotropin-releasing hormone (CRH) released from the hypothalamus plays important roles in regulating stress through the hypothalamic–pituitary–adrenal (HPA) axis (De Souza, 1995). Hypothalamic CRH is released to the hypophyseal portal vessel and stimulates the release of adrenocorticotropic hormone (ACTH) from corticotrophs in the anterior pituitary. Then ACTH stimulates glucocorticoids release from the adrenal cortex to cope with the stressors. In the autonomic nervous system, sympathetic nervous system tone is increased by stressor exposure, and it induces various responses via the releases of norepinephrine (NE) from the post-ganglionic terminals and epinephrine (E) from the adrenal medulla. Both NE and E exert their effect through adrenergic receptors which include several subtypes such as alpha-1-, alpha-2, and beta-receptors (Bentley, 1998).

It is well known that stressors induce dysfunction of the digestive tract such as the induction of gastrointestinal ulcers, alteration of gastrointestinal motility, and alteration of food passage (Caso et al., 2008). Among them, the alteration of food passage is thought to be partly induced by stress-related hormones in mammals (Caso et al., 2008). For example, central injection of CRH inhibits gastric emptying in dogs, rats, and mice (Bueno and Fioramonti, 1986; Taché et al., 1987; Martínez et al., 2004), and the effect of CRH is at least partly mediated by corticosterone (CORT), a glucocorticoid (Broccardo and Improta, 1990). Urocortins (UCNs), members of the CRH-family and bind to CRH receptor, also inhibit gastric emptying in mice (Martínez et al., 2004). Moreover, NE, E and their receptor agonists have been demonstrated to reduce the rate of food passage from the stomach and small intestine in rodents (Gáti et al., 1975; Tsukada et al., 2003). Studies using adrenergic receptor agonists also suggest that both alpha- and beta-adrenergic receptors are related to the effects of NE and E (Ruwart et al., 1980; Liberge and Bueno, 1989; Asai et al., 1997a,c). Collectively, these stress-related hormones contribute to the delay of food passage from the digestive tract of mammals.

In chickens, CRH has been identified, and its amino acid sequence is the same as rodents and human (Vandenborne et al., 2005). Furthermore, the physiological effects of CRH in chicks are similar to mammals. For example, intracerebroventricular (ICV) injection of CRH inhibits food passage in the crop, which is situated

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at the esophagus in the avian (Ogino et al., 2014). This inhibitory effect is also observed when UCN3, a member of UCNs, is ICV injected (Ogino et al., 2014), suggesting that HPA axis is related to the inhibition of food passage from the digestive tract in avian. Indeed, intramuscular injection of CORT every other day reduces food passage from the digestive tract in 8- and 15-day-old chicks (Tur et al., 1989). However, the acute effect of CORT on food passage is still unknown. Moreover, whether NE and E affect food passage from the digestive tract has not been well documented in chicks.

In the present study, we investigated the effect of acute injections of CORT, NE and E on food passage in the crop, which is located at the end of the esophagus (Denbow, 2000). Furthermore, which receptor subtypes were related to the effects of NE and E was also investigated using agonists. Finally, the number of defecations after injection of CORT, NE, and E was investigated. The effect of ICV injection of CRH and UCN3, on the number of excreta was also examined in order to investigate the relationship between CORT and CRH and UCN3.

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 (1996). This study was approved by the Committee of Animal Care and Use in Ehime University (No. 08-o3-10).

2.2. Drugs and injection

All injections were made between 0800 and 1000, because the blood concentration of CORT and catecholamine is different between daytime and night (De Jong et al., 2001). CORT (Wako Pure Chemical Industries, Osaka, Japan) was dissolved in dimethyl sulfoxide (DMSO), and then diluted with polyethylene glycol so as to be 5% DMSO. This CORT solution was served for subcutaneous (SQ) injection via the inner thigh at a volume of 0.1 ml. The vehicle was used for the control treatment. The injection route was based on a previous study using glucocorticoid in chicks (Song et al., 2011).

NE hydrochloride (Sigma Aldrich, St. Louis, MO, USA), E hydrochloride, phenylephrine hydrochloride (PNL, an alpha-1-adrenergic receptor agonist), clonidine hydrochloride (CLON, an alpha-2-adrenergic receptor agonist, Wako Pure Chemical Industries, Osaka, Japan), and isoproterenol hydrochloride (ISO, a non-selective beta-adrenergic receptor agonist, Tokyo Chemical Industry, Tokyo, Japan) were dissolved in a normal saline solution and the vehicle alone was used for the control treatment. These solutions were injected via the intraperitoneal (IP) route at a volume of 0.2 ml per chick. This injection route was based on reports in mammals (Liberge and Bueno, 1989; Asai et al., 1997a,c).

Chicken CRH (Peptide Institute, Osaka, Japan) and human UCN3 (92% homology with chicken, Phoenix Pharmaceuticals, Burlingame, CA, USA) were dissolved in a normal saline solution containing 0.1% Evans Blue dye. For UCN3, dimethyl sulfoxide was added at 5% total volume to aid in dissolution. The vehicle only was used for their control treatment. Solutions were administered to chicks by ICV injection because these peptides are expressed in the brain. ICV injections were performed according to a previously reported method (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. The injection procedure is rapid and does not result in additional stress to chicks judging from food intake and corticosterone release data (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 show the presence of Evans Blue dye in the lateral ventricle were not used for analyses.

2.3. Effect of CORT on crop emptying

Crop-emptying rate was measured based on the method previously reported (Tachibana et al., 2010). Seven-day-old chicks (mean body weight: 48.8 ± 0.3 g), which were food-deprived for 15 h to empty residual ingesta within the crop, were SQ injected with vehicle (control), 0.9 or 3.5 μmol CORT. 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. Sixty or 120 min after the gavage, chicks were deeply anesthetized by inhalation of diethyl ether, after which their crops were exposed, the upper and lower esophagus clamped and the crop excised. Total content of the crops were recovered and 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.

2.4. Effects of NE and E on crop emptying

Eight-day-old chicks (mean body weight: 52.9 ± 0.4 g) were food-deprived for 15 h and then IP injected with vehicle (control), 60 or 240 nmol NE. Chicks were gavaged with a feed slurry as noted above. Sixty or 120 min after the gavage, chicks were deeply anesthetized by inhalation of diethyl ether, and thereafter the crop contents were obtained, dried and weighed for the calculation of the crop emptying rate.

The E study was performed using 7-day-old chicks (mean body weight: 48.5 ± 0.3 g) and the procedures were the identical to the NE study.

2.5. Effects of PNL, CLON, and ISO on crop emptying

Eight-day-old chicks (mean body weight: 48.8 ± 0.5 g) were food-deprived for 15 h and then IP injected with vehicle (control), 60 or 240 nmol PNL. Immediately after the injection, chicks were gavaged with a feed slurry as aforementioned. Chicks were deeply anesthetized by diethyl ether at 120 min after the gavage, and thereafter the crop content was obtained, dried and weighed for the calculation of the crop emptying rate.