Domestic Animal Endocrinology xxx (2018) 1–7

Contents lists available at ScienceDirect

Domestic Animal Endocrinology

journal homepage: www.journals.elsevier.com/domestic-animal-endocrinology

Effect of L-tryptophan and its metabolites on food passage from the crop in chicks

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A R T I C L E I N F O

Article history:
Received 8 November 2017
Received in revised form 9 January 2018
Accepted 11 March 2018

Keywords:
5-Hydroxytryptamine
Chick
Crop emptying
Melatonin
Tryptophan

A B S T R A C T

L-tryptophan (L-Trp), an essential amino acid, is well known as a precursor of 5-hydroxytryptamine (5-HT) and melatonin. In mammals, L-Trp itself has been reported to suppress gastric emptying in mammals. In addition, 5-HT and melatonin are found in the gastrointestinal tract and affect food passage from the digestive tract in mammals. While the function of these factors in mammals is documented, there is little knowledge on their function in the digestive tract of birds. Therefore, the purpose of the present study was to determine if L-Trp and its metabolites affect the crop emptying rate in chicks (*Gallus gallus*). We also investigated the effects of kynurenic acid (KYNA) and quinolinic acid (QA), which are metabolites of the kynurenine pathway for L-Trp. Oral administration of L-Trp significantly reduced the crop emptying rate in chicks. Among the metabolites, intraperitoneal injection of 5-HT and melatonin significantly reduced the crop emptying rate, whereas KYNA and QA had no effect. The present study suggested that L-Trp, 5-HT, and melatonin inhibit the movement of food in the digestive tract and thereby affect the utilization of nutrients in the diet of chicks.

1. Introduction

The digestive system is the primary organ for obtaining nutrients from the diet, so this organ is pivotal in achieving optimal performance in poultry production [1]. The functions of the digestive system are immature in neonatal chicks (*Gallus gallus*) [2,3]; so early development of the digestive function is thought to enable better utilization of nutrients and efficient growth [3]. Although many factors such as diet composition affect the function of the digestive system, the mechanism underlying the regulation of the digestive system has not been fully clarified in neonatal chicks.

L-tryptophan (L-Trp) is an aromatic amino acid and is used in the biosynthesis of protein. In addition, L-Trp is used as a precursor of several bioactive molecules including 5-hydroxytryptamine (5-HT, serotonin) and melatonin. 5-hydroxytryptamine is a monoamine neurotransmitter with a diverse array of actions including regulation of food intake [4] and sleep-wake behavior [5]. Melatonin is also involved in many physiological processes including regulation of circadian rhythms [6]. Both 5-HT and melatonin exist in the gastrointestinal tract [7–10] and affect food passage in the digestive tract in mammals. For example, intraperitoneal (IP) injection of DL-fenfluramine, a 5-HT receptor agonist, decreases gastric emptying in rats [11], and antagonism of the 5-HT3 receptor accelerates gastric emptying in rats [12]. In addition, glucose-induced inhibition of gastric emptying is abolished by antagonism of the 5-HT3 receptor in rats [13]. Intraperitoneal injection of melatonin reduces the gastric emptying rate via a mechanism related to cholecystokinin (CCK) and 5-HT in rats [14]. Furthermore, L-Trp has been reported to suppress gastric emptying in dogs, whereas other amino acids such as

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https://doi.org/10.1016/j.domaniend.2018.03.001

glycine (Gly), asparagine (Asn), alanine (Ala), and serine have no effect [15–17]. These results demonstrate that L-Trp and its metabolites are related to the inhibition of gastric emptying in mammals.

5-hydroxytryptamine and melatonin are also distributed in the digestive tract of nonmammalian vertebrates [10,18]. For example, in chickens, both 5-HT and melatonin are found in the digestive tract [19], but little is known about their physiological role. Although 5-HT has been demonstrated to contract the esophagus of young chickens in vitro [20] and to suppress gastric secretion in chickens [21], the effect of 5-HT on food passage in the digestive tract remains unstudied. To our knowledge, the effects of L-Trp and melatonin on the digestive tract have not been reported in chickens. Furuse et al [22] demonstrated that L-phenylalanine (L-Phe), another essential amino acid, suppresses the crop emptying rate in young chickens. This result suggests that other amino acids including L-Trp itself may be involved in regulating food passage in the digestive tract in chickens. In addition, it is also possible that the metabolites of L-Trp, such as 5-HT and melatonin, affect food passage in the digestive tract in chickens, as has been shown in mammals.

The purpose of the present study was to investigate whether L-Trp, 5-HT, and melatonin affect food passage from the crop in chicks (*G gallus*). First, we investigated the effect of oral administration of L-Trp and other amino acids on the crop emptying rate. Subsequently, the effect of IP injection of L-Trp, 5-HT, and melatonin on the crop emptying rate was investigated to clarify the mechanism underlying the effect of orally-injected L-Trp. We also investigated the effect of kynurenic acid (KYNA) and quinolinic acid (QA), which are the metabolites produced from the kynurenine pathway of L-Trp [23,24], on the crop emptying rate.

2. Materials and methods

2.1. Animals

Day-old male layer chicks (*G gallus*, white leghorn, Julia) were purchased from a local hatchery (Nihon Layer, Gifu, Japan) and raised in a room kept at 30°C with continuous lighting. A commercial diet (crude protein: 24%, ME: 3,050 kcal/kg; Toyohashi Feed Mills Co Ltd, Aichi, Japan) and water were available ad libitum to the chicks. Chicks were transferred to an experimental cage at least 2 d before each experiment to accustom them to the experimental conditions. They were individually caged 1 d before each experiment. Before the experiment, BW was measured and then the chicks were distributed into experimental groups so that the average BW was as uniform as possible between the treatment groups. The chicks were maintained in accordance with the recommendations of the National Research Council [25]. This study was approved by the Committee of Animal Care and Use of Ehime University, Japan (No. 08-o3-10).

2.2. Drugs and injections

L-tryptophan, 5-HT creatinine sulfate, KYNA (Sigma-Aldrich, MO), Gly, L-Asn, QA, melatonin (Wako Pure Chemical, Osaka, Japan), and L-Ala (Kanto Chemical, Tokyo, Japan) were used in the present study. These drugs were used for IP injection although L-Trp was additionally used for oral injection.

For oral injection, L-Trp and other amino acids were suspended in distilled water and then mixed with a powdered diet as described in the following. For IP injection, L-Trp and 5-HT were dissolved in a normal saline solution. Melatonin and QA were first dissolved in dimethyl sulfoxide and then diluted with the saline so that the concentration of dimethyl sulfoxide was 5%. Kynurenic acid was first dissolved in 0.1 M sodium hydroxide and then diluted with 0.01 M PBS (pH 7.4). Vehicle only was used as the control treatment. These solutions were injected into the abdominal cavity at a volume of 0.2 mL per chick. All Q2 injections were performed between 8 AM and 10 AM.

2.3. Crop emptying rate

The crop emptying rate was determined based on a previously reported method [26]. Chicks, which were food deprived for 15 h (to empty residual ingesta within the crop), were gavaged with a feed slurry into the crop at a mass of about 4% BW. The feed slurry was made by mixing 40% powdered diet with 60% distilled water on a weight basis. No chicks vomited after gavage in the present study. After gavage, chicks were returned to the individual cages, and feed and water were withheld. At 1 or 2 h after the gavage, the chicks were euthanized by inhaling carbon dioxide, after which their crops were exposed, the upper and lower esophagus clamped, and the crop excised. The total content of the crops was 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. The wet slurry weight was then calculated based on the dry weight. The weight of the slurry emptied from the crop through the lower esophagus was calculated by subtracting the weight of the slurry within the crop from the weight of the administered slurry. The crop emptying rate was expressed as the percentage of slurry emptied from the crop to the amount gavaged.

2.4. Effect of oral injection of L-Trp on the crop emptying rate

For this experiment, L-Trp was administered by dissolving it in the feed slurry. Five-day-old chicks fasted for 15 h were gavaged with the feed slurry, which contained 0 (control), 54, or 215 μmol L-Trp, into the crop. The crop emptying rate was calculated as described previously. The dose of L-Trp was decided based on a mammalian study [16].

In a time-course study, 6-day-old chicks fasted for 15 h were placed into the control and 215 μmol L-Trp groups, and further separated into the 1 h or 2 h groups. They were gavaged with a feed slurry containing 0 (control) or 215 μmol L-Trp. The crop emptying rate was calculated as described previously.

2.5. Effect of oral injection of Gly, L-Ala, and L-Asn on the crop emptying rate

All amino acids were injected by dissolving in the feed slurry as described in the L-Trp study. Six-day-old chicks,