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Purinergic Regulation of Food and Fat Intakes in Broiler's Central Nervous System

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Abstract

BACKGROUND: Adenosine has many physiological roles in the brain, and in rodents, it changes food intake when applied centrally.

OBJECTIVES: We investigated the effect of central injection of the purine molecule adenosine on both food and fat intakes in neonatal chicks.

METHODS: In the first trial, various doses of adenosine (an endogenous P1 receptor agonist), and its synthetic antagonist CGS-15943, were injected intracerebroventricularly (ICV) to the chicks and the cumulative food intake was measured at definite time intervals. The second trial was similar to the first one, only the chicks were fed with a high-fat diet.

RESULTS: Adenosine did not affect food or fat intake. Food consumption was increased 30 min after injection of CGS-15943. CGS-15943 also increased fat intake in chicks fed a high-fat diet.

CONCLUSIONS: The present study suggests that in the avian central nervous system, P1 receptors are entailed in the regulation of food and fat intake in an antagonistic manner.

KEYWORDS: Adenosine, Brain, Broilers, Fat intake, Food intake

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Introduction

There are some necessary ligands, including hormones, neuropeptides, and other signaling molecules, for the central regulation of food intake in birds (Denbow & cline, 2015) and mammals (Morton *et al.*, 2014). During broiler chickens' growing period, the body weight gain is important to calculate the growth and feed conversion ratio (Fontana *et al.*, 2017). The mechanisms underlying food intake control in neonatal chicks have been extensively studied during the last decade. Birds look for food spontaneously just after hatching. There are differences in the functions of signaling molecules that regulate food intake in birds and mammals so that some of them, such as ghrelin, peptide YY, and pancreatic polypeptide, act quite oppositely in birds (Denbow & cline, 2015). Understanding fat intake regulating systems is also important in birds, either in commercial poultry farms or in flying birds. In broiler strains, as the aim is to get high meat production in a short period, overeating causes increased fat tissue and consequent adverse health effects such as leg diseases, reduced reproductive efficiency, etc. In flying birds, also, fat accumulation and overweighting are not appropriate (Honda *et al.*, 2017).

Purinergic receptors are widespread throughout the central nervous system (CNS) and are involved in the modulation of many neural systems (Burnstock, 2020), such as feeding behavior (Ciruela & Jacobson, 2019).

Adenosine triphosphate (ATP) and adenosine diphosphate (ADP) are intracellular metabolites that can also act as extracellular transmitters via different classes of P2 receptors. After release, these molecules are degraded to adenosine. This metabolite is also important as it is a physiologic ligand of P1 receptors in the brain, which includes A1, A2A, A2B, and A3. Adenosine releases in conditions such as metabolic stress, tissue injury, hypoxia, and inflammation (Fredholm, 2014).

In comparison to humans and rodents, the effects of the purinergic system on food intake in birds have not been extensively studied (Burnstock, 2020). In a recent study, we showed that ATP increased food intake in broilers (Motaghi *et al.*, 2019). As there is no report about the central role of adenosine in birds, we investigated the effect of intracerebroventricular (ICV) injection of adenosine and its antagonist on food and fat intake in neonatal chicks.

Materials and Methods

Birds and the Experiment Design

One-day-old Ross broiler chicks were obtained from a local hatchery (Mahan Chicken Meat Production Complex, Kerman, Iran) and reared as a group for four days in electrically heated batteries (24 h lighting, 31±1°C temperature, and humidity of 50% to 60%). Two days before injections, the chicks were transferred to single cages with food and water ad libitum. The injections were done at six days of age to ensure that the yolk sac was absorbed. All injections were done ICV based on our previous experiments (Motaghi *et al*., 2017, 2019).

Briefly, the head of each chick was fixed in a restraining device, which held the beak at the angle of 45°; thus, the skull was parallel to the surface of the table. There was a plate on this device over the skull with a hole over the right lateral ventricle so that a microsyringe needle could be inserted into the ventricle through this hole. The tip of the needle penetrated 4 mm below the skin of the skull. This procedure causes no physiological stress in neonatal chicks. The volume of injection was 5 µL. Before ICV injections, in order to synchronize feeding, the birds were deprived of food for 3 h. After injections, the feed was given freely, and cumulative food intake was measured at 30, 60, 120, and 180 min after injection. All the procedures, carried out in this study, were confirmed by the Animal Ethics Committee of Shahid Bahonar University of Kerman.

Treatments

All drugs were purchased from Tocris Bioscience (Bristol, UK). In experiment 1, adenosine, a P1 receptor endogenous agonist (0, 10, 50, and 100 µg), was dissolved in 20% DMSO plus 0.1% Evans blue and injected ICV into each chick. The Evans blue made the solutions chromatic, acted as a marker to verify the injection of solutions into the right ventricle. Chicks were 9-11 per group. In experiment 2, CGS-15943, a potent non-specific synthetic adenosine receptor antagonist (0, 0.75, 1.5, 3, 5, 10, and 20 µg), was used. The control/solvent solution for CGS-15943 was made up of 12.5% propylene glycol,

12.5% castor oil, and 25% DMSO plus 0.1% Evans blue.

For fat intake studies, based on the obtained results from experiments 1 and 2, adenosine (0, 10, and 50 µg) and CGS-15943 (0, 5, and 10 µg) were injected into the broilers. The procedures were like before, but the differences were as follows: the chicks were fed a normal diet (Table 1) similar to the last experiments from day 0 to 24 h later. Then, 4% fat was added to the diet on day 2. On days 3 and 4, the fat in the diet increased to 7% and 11%, respectively (Table 2). From day 4 up to the end of the experiments, the percentage of fat was 11%, so that after the injections, the chicks were fed this diet. The gradual increase of fat in the diet was for better adaptation of broilers to a high-fat diet.

After gaining the required data, the chicks were euthanized with sodium thiopental. Then, the brains were removed, and data from chicks, in which the dye was not present in their lateral ventricle, were omitted.

Statistical Analysis

Cumulative food intake (as a percentage of body weight) was calculated for each chicken, which means that for acquiring cumulative food intake, the amount of eaten food body weight multiplied by 100. Data were analyzed using SPSS 22 (SPSS Inc., Chicago, Ill., USA). We used one-way analysis of variance (ANOVA). The significant effect was detected by the Duncan post hoc test. A significant effect was reported when *P*-values were less than 0.05. Data are presented as mean±SEM.

Table 1. Components of normal food diet

Energy	Crude protein	Methionine	Cysteine	Lysine	tryptophan	Absorable Phosphorus	Ca	Na
2 850 Kcal/kg	23%	0.52%	0.85%	1.3%	0.3%	0.5%	1%	**0.18%**

Table 2. Components of fat diet

Fat (%)	Corn (%)	Corn oil (%)	Ca (co3)2 (%)	Ca2(PO4) (%)	Methionine (%)	NaCl (%)	Vitamins& minerals (%)	Soy meal (%)
4	53.5	0.8	1.7	1.4	0.2	0.4	0.2	41.8
7	48.6	4.7	1.8	1.4	0.2	0.4	0.2	42.7
11	43.3	9.1	1.8	1.4	0.2	0.4	0.2	43.6

Results

Food Intake Studies

ICV injection of a wide range of adenosine from 10 to 100 µg did not change cumulative food intake in the mentioned time intervals (Figure 1). CGS-15943 increased food intake at 5 µg from 30 to 120 min after injection ($P \leq 0.05$; Figure 2). These results show that CGS-15943, in a restricted range (i.e., 5 µg), can increase food intake. This increase reached significance at a dose of 10 µg in the mentioned period ($P < 0.05$; Figure 2).

Fat Intake Studies

In fat intake studies, we administered 10 and 50 µg of adenosine. Figure 3 shows that in comparison with the control group, the lower dose tends to decrease fat intake, but the higher one increases it. Both effects did not reach significance, but at 60 min after injection, these contradictory effects reached significance with each other ($P \leq 0.05$; Figure 3). CGS-15943 at a dose of 10 µg increased fat intake at 30 and 120 min after injections ($P \leq 0.05$; Figure 4).

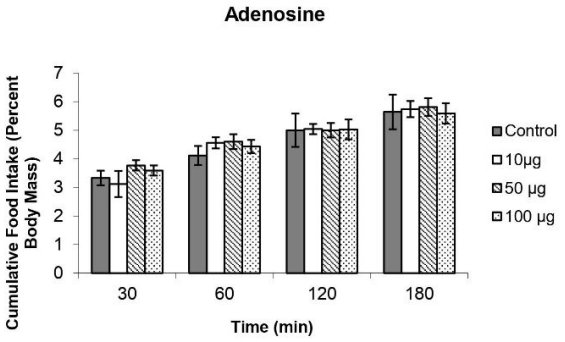

Figure 1. Effect of intracerebroventriculalr administration of adenosine (10, 50 and 100 µg) on food intake. Data is shown as mean ± SEM. $P \leq 0.05$ considered significant. Post hoc test: Duncan.

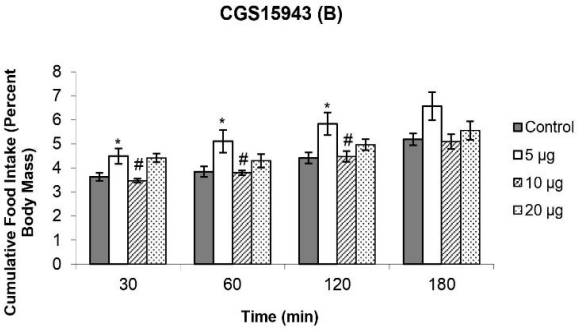

Figure 2. Effect of intracerebroventriculalr administration of CGS15943 (5, 10 and 20 µg) on food intake. Data is shown as mean ± SEM. $P \leq 0.05$ considered significant. Post hoc test: Duncan * $P \leq 0.05$ in comparison with the control group. # $P \leq 0.05$ in comparison with the group which received 5µg of CGS15943.

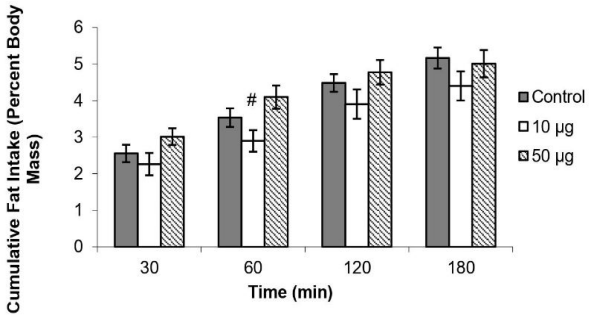

Figure 3. Effect of intracerebroventriculalr administration of adenosine (10 and 50 µg) on fat intake. Data is shown as mean ± SEM. $P \leq 0.05$ considered significant. Post hoc test: Duncan. # $P \leq 0.05$ in comparison with the group which received 50µg of adenosine.

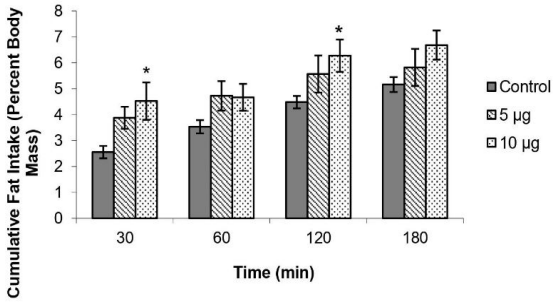

Figure 4. Effect of intracerebroventriculalr administration of CGS15943 (5 and 10 µg) on fat intake. Data is shown as mean ± SEM. $P \leq 0.05$ considered significant. * $P \leq 0.05$ in comparison with the control group.

Discussion

Our results showed that CGS-15943 increased food and fat intake. Adenosine in the brain originates from ATP. ATP in the brain originates from neuron terminals and also astrocytes. After release, it is metabolized by several families of ectonucleotidases. The final metabolites are adenosine monophosphate (AMP), ADP, or adenosine (Burnstock, 2020).

In this study, adenosine did not alter either cumulative food or fat intake in chicks (Figures 1 and 3). However, in a study by Levine and Morely (1983), food intake was suppressed in rodents by adenosine 30 min after ICV injection. In another study, synthetic agonists of adenosine increased food intake in rats (Levine *et al.*, 1989). In agreement with our results, in a study by Prichett *et al.* (2010) in rodents, fat intake was not changed by intra accumbance administration of the selective agonist of adenosine receptors (A1 and A2A).

Adenosine increases intracellularly when there is an imbalance between the rate of ATP production and the rate of ATP consumption; in this case, ATP levels decrease, and, consequently, adenosine levels increase. Intracellularly formed adenosine would then, employing its transporters, exit cells and act on receptors in the local environment. Adenosine can enter the brain tissue. Via adenosine A1 receptors,

adenosine has been shown to reduce the activity of orexinergic neurons (Thakkar *et al.*, 2008), basal forebrain cholinergic neurons (Rainnie *et al.*, 1994), and locus coeruleus noradrenergic and pontine serotoninergic neurons. All of these investigations reported reduced turnover or release of these transmitters. The mentioned studies show that the central adenosine may control feeding behavior (Honda *et al.*, 2017).

In this study, it is not easy to interpret the results of this study. Levine and Morely (1983) indicated that central administration of inosine (an adenosine deamination metabolite) did not affect food intake, so maybe adenosine was degraded to other metabolites after ICV injection and thus did not affect food intake.

Injection of CGS-15943, a non-specific antagonist of adenosine receptors, increased both food and fat intakes (Figures 2 and 4). In this regard, William *et al.* (1987) reported that CGS-15943 in the range of 0.85 to 5.71 µg inhibited adenosine receptors. In our study, also, food intake increased at 5 µg, the same range of inhibiting adenosine receptors *in vitro*. In another study by Yang *et al.* (2015), intra-arcuate nucleus injection of a selective adenosine A1 receptor antagonist (DPCPX) just transiently caused hyperphagia within 30 min after injection. Another study also showed that intra-nucleus accumbens (NAcc) injection of MSX-3, a selective antagonist of A2A receptor, increased fat intake, as mentioned before, similar to our study; these authors also did not observe any change in fat intake by adenosine (Pritchett *et al.*, 2010).

NAcc plays a central role in both appetitive goal-seeking and consummatory behaviors for food or drugs of abuse. Injections of A2A receptor agonists into the NAcc reduced lever pressing for a preferred food reward and promoted a switch to enhanced consumption of freely available but less preferred food (Mingote *et al*., 2008). Such data suggest that NAcc A2A receptors are important in regulating food-directed reward-seeking behaviors. The effect of accumbal A2A receptors on reward-driven feeding in a sated state was confirmed by the facilitatory effect of A2A receptor inhibition on opioid-driven intake of a high-fat diet (Pritchett *et al.*, 2010). Some reports have demonstrated that intra-NAcc injections of a µ-opioid agonist increase the consumption of a high-fat diet in sated animals (Parker *et al.*, 2010), indicating a role for opioid receptors in promoting food intake beyond homeostatic levels. Additionally, microinjections of opioid receptor agonists directly into the NAcc increased the rats' efforts to gain access to a food reward (Zhang *et al.*, 2003).

Thus, there are both physiological and behavioral data to suggest that µ-opioid and A2A receptors within the NAcc are important in regulating food-directed reward-seeking behaviors. In the end, the present study suggests that P1 receptors act in an antagonistic and dose-dependent manner in the chick brain to regulate food and fat intake. To the authors' knowledge, it is the first study on the role of adenosine signaling in the regulation of behaviors in avian species.

Conclusion

None.

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Conflict of Interest

There were no conflict of interest among authors.

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تنظیم پورینرژیک اخذ غذا و چربی در سیستم عصبی مرکزی جوجه‌های گوشتی نوزاد

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(دریافت مقاله: ۲۰ بهمن ماه ۱۳۹۹، پذیرش نهایی: ۱۳ اردیبهشت ماه ۱۴۰۰)

زمینه مطالعه: آدنوزین دارای نقش‌های متعدد فیزیولوژیک در مغز است. تجویز این ماده در مغز جوندگان اخذ غذا را تغییر داد.

هدف: از مطالعه حاضر، بررسی اثر تجویز داخل مغزی مولکول پورینی آدنوزین، بر اخذ غذا و چربی، در جوجه‌های گوشتی نوزاد بود

روش کار: در آزمایش اول دوزهای مختلف آدنوزین و CGS15943 که به ترتیب آگونیست درون زاد گیرنده‌های P و آنتاگونیست مصنوعی و غیر اختصاصی آن بودند، به‌صورت داخل بطن مغزی به جوجه ها تجویز شد و اخذ غذا در زمان‌های معین بعد از تجویز اندازه‌گیری شد. آزمایش دوم مشابه قبل بود با این تفاوت که جوجه ها با رژیم غذایی حاوی چربی بالا تغذیه شدند.

نتایج: آدنوزین اثری بر اخذ غذا و چربی نداشت. اخذ غذا ۳۰ دقیقه بعد از تجویز CGS15943 افزایش پیدا کرد. CGS15943 اخذ چربی را از ۳۰ تا ۱۲۰ دقیقه بعد از تزریق، در جوجه‌هایی که از رژیم با چربی بالا استفاده کرده بودند، افزایش داد.

نتیجه‌گیری نهایی: در سیستم عصبی مرکزی پرندگان، آدنوزین اخذ غذا و چربی را با روندی آنتاگونیستی تنظیم می‌کند.

واژه‌های کلیدی: آدنوزین، اخذ غذا، اخذ چربی، جوجه‌های گوشتی، مغز

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