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Research Article
Effect of Central Adrenomedullin on Behavioral Changes in the Japanese Quail

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Abstract

Background and Objective: Adrenomedullin (AM), primarily extracted from the adrenal medulla of humans, contains 52 amino acids. It is reported to have a powerful hypotensive and anorexic effect on mammals. This study aimed to clarify the role of AM in avian species as an alternative vertebrate model. **Materials and Methods:** Adult male Japanese quail (*Coturnix japonica*) were assigned into 4 groups; besides the control (vehicle) group, AM was administrated intracerebroventricular (ICV) with doses of 0.1, 0.5 and 1.0 nmol/10 µL saline to determine its influence on food and water intake, body temperature and gross locomotor activity. Moreover, immunohistochemical staining was conducted to determine the effect of AM on changes in hypothalamic chemistry. Also, another trial was conducted to detect the effect of astressin as a corticotropin-releasing hormone antagonist on AM. **Results:** The quails centrally injected with AM had reduced body weight and food intake dose-dependently at 2, 4 and 8 hrs after injection, but not water intake, increases in locomotor activities and transient non-significant elevation in body temperature. Japanese quails that received central AM showed an increase in c-Fos immunoreactivity in the dorsomedial hypothalamus (DM), ventromedial hypothalamus (VMH) and paraventricular Magnocellular (PaMC). The paraventricular parvocellular (PaPc) and arcuate nucleus (ARC) were significantly influenced, while the lateral hypothalamus (LH) was not significantly affected. Receptors' antagonistic effect of corticotrophin-releasing factor (CRF) showed no changes in AM-associated anorexia. **Conclusion:** Exogenous human AM-ICV administration has a CRF receptor non-dependent anorexigenic effect on locomotor activity and transient body temperature. Japanese quail exhibits a change in hypothalamic chemistry along with activation of VMH, DM and PaPC.

Key words: Adrenomedullin, behavioral changes, intracerebroventricular, Japanese quail, hypothalamic chemistry, astressin

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

In the human adrenal medulla, adrenomedullin (AM) is a regulatory peptide with strong hypotensive effects that have been described by Kitamura *et al.*[1]. Since its discovery, numerous studies have assured its expression in almost every tissue, but most commonly in the heart, kidney, pancreas, intestines and lungs[2-5]. Through, human adrenomedullin, RNA-blot analysis for mRNA has shown it is widely expressed in most body tissues with increased concentration in fat cells, lungs, smooth muscle, pancreatic islets, skin and placenta[6,7]. The wide distribution of AM usually accompanies broad biological action ranges; it includes regulatory hormone secretion, cell growth, vasodilatation and antimicrobial effects[8-10].

Chemically speaking, AM has a single intramolecular disulfide bond and shows some slight resemblance to amylin, Adrenomedullin-2/Intermedin (AM2/IMD) and CGRP[11,12]. According to several publications, the receptor activity-modifying protein (RAMP)/Calcitonin Receptor-like Receptor (CRLR) is activated by Calcitonin-gen Related Peptide (CGRP), AM and AM2/IMD[13-15]. Also, AM receptor mRNA can be found predominantly in the spleen, lung, ovary and adrenal glands. Still, it is found in rat's cerebellum and cerebral cortex at scanty levels. The CGRP neuropeptides family influences appetite in various vertebrates. Rodents and goldfish were reported to be negatively impacted by peripheral and central administrations of CGRP, AM2/IMD and amylin[16-20]. Similar effects were noticed in chicks[21,22]. Thus, rats can experience anorexic effects from central AM. Some authors attributed this effect to the action of AM on CGRP receptors[23,24]. Though, AM is accompanied by complete satiety in rats, it does not influence the food intake level of goldfish[19].

Rat Paraventricular Nuclei (PVN) and supraoptic nuclei (SON) express c-Fos in response to central AM administration, particularly in the magnocellular portions[25,26]. Other studies stated that neuropeptides cause decreased appetite via corticotrophin-releasing hormone[27,28]. Despite the proven effect of AM on appetite and food consumption in humans and rats, little data are available about AM's effect on the avian species. Therefore, this study aimed to cover the unmet data about the possible effect of central human AM-ICV on adult Japanese quail as an avian model because the adult Japanese quail showed little fluctuation in feed consumption and body weight compared to chicks, which showed a steep growth curve. Thus, the effect of AM can easily and accurately be monitored, besides the technical possibility of implanting an ICV cannula into adult Japanese quail. In this study, the impact of AM-ICV on physiological changes, like food intake, water intake, body gain, body temperature, locomotor activities and immune responses were examined. The study also determined whether possible AM-induced satiety depends on CRF and examined which appetitive hypothalamic nuclei are most sensitive to central AM.

MATERIALS AND METHODS

Study area: This experiment was performed in the Department of Veterinary Physiology, Faculty of Agriculture, University of Miyazaki, Japan from March to September, 2017.

Experimental animals: The 24 Japanese quail (*Coturnix japonica*) were obtained from a local farm in Kibana Dai Nishi, Miyazaki, Japan; the adult males were housed individually in net cages measuring 14/26/17 cm in a room that was 28°C with a 12 hrs light (300 lux) to 12 hrs dark (dim light, 25 Lux) cycle. The lights were turned on at 7:00 am. The birds had unrestricted access to food and water. The birds were weighed before the experiment and six birds were placed in each experimental group according to their body weight. Each group's average body weight (110-120 g) was maintained as consistently as feasible. Human adrenomedullin (AM, 6028.9 molecular weight, Peptide Institute, Osaka, Japan) or 0.9% saline (vehicle control) was supplied ICV at 10 hrs to study the impact of adrenomedullin (AM) on eating regulation in Japanese quail. The doses administered were 0.1, 0.5 and 1.0 nmol/10 µL saline. The same animals were not used for more than one study. Every action was taken in accordance with the standards of the Japanese Physiological Society for Animal Care.

Intracerebroventricular (ICV) injection procedures: For inserting the ICV cannula, 5% sodium pentobarbital (1.4 µL/g b.wt.) was used for the bird's anesthetization and each anaesthetized bird was located in a stereotaxic casing. A guiding cannula made of stainless steel (diameter 550 µm, length 14 mm) was implanted stereotaxically into the third cerebral ventricle using a modification of a previously reported method by Zendehdel *et al.*[29]. The coordinate lines were 6.5 mm below the dura at the midline and 5 mm anterior to the interaural axis. The guiding cannula was cemented with acrylic dental cement and an anchoring stainless steel screw was fastened to the skull. The birds were given at least a 4 day healing time before being placed back in their separate cages. They were accustomed to handling every day before the

beginning of the experiments. An implanted cannulae was used for the ICV administrations without anesthesia or bird restraining. After data collection, administration of 10 µL of Evans Blue dye, followed by bird sacrificing and sectioning of the brain at 20 µ intervals, were applied to verify the proper settlement of the cannulae. The birds that showed no dye in the third ventricle were omitted from the statistical analysis.

AM's impact on dietary intake and hydration: After a 3 hrs fast, the tested birds were allowed free access to food and water after returning to their respective cages. At 2, 4 and 8 hrs after AM or saline treatment, food consumption was quantified (to an accuracy of 0.01 g) by observing the drop in food troughs. A digital balance with a 0.001 g precision was used to measure the water loss from the water cup (pre-weighted) to estimate water intake at 30, 60, 120, 150 and 180 min following peptide delivery. Additionally, the amount of water evaporation throughout the experiment was measured and the results were utilized to adjust the amount of water consumed. Additionally, errors were minimized by the uniform distribution of the control and experimental groups in the experimental room.

AM's impact on body temperature: After injection, the quails' body temperatures were measured at 5, 10, 20, 40, 60 and 120 min later using a previously described technique by Zendehdel *et al.*[29]. The AM or saline was given at the before mentioned dosages (n = 6 in each group) at 10 hrs, to monitor body on the outside, a tiny sensor with a measurement range of 25 to 50°C and a measurement error of 0.05°C was used to electronically detect the temperature. After inserting the sensor tip into the cloacae and attaching a section of the line to the bird's body, the monitor body received the digital signal.

AM's impact on overall motor activity: Birds were located in their individual cages with auditory but not visual contact with each other. The locomotor activity was grossly measured under LD conditions in each bird for 1 week and thereafter. The activity was estimated under continual dim light with an intensity of about 30 lux, using a rat locomotor activity recording system (Muromachi, Tokyo, Japan) involving infrared sensors, an interface and a computer[30]. To track locomotor behaviors like feeding and moving inside the cages, infrared sensors were placed above the cages. The infrared sensor-equipped cages were kept apart in an insulated chamber box with a predetermined cycle of light and dark. The data were gathered at 15 min intervals and Compact ACT AMS software (Muromachi) was used for data analysis. The dosages of 0.1, 0.5 and 1.0 nmol/10 µL saline were given to the birds in the groups (n = 6 per group) at 10 hrs. The separate cages where the birds were kept were immediately opened. The locomotor activity counts were assessed every 15 min and continued for 2 hrs following treatment.

Indicator of neuronal activity by AM: The experimental birds were randomly assigned to receive a vehicle or an ICV injection of 1.0 nmol AM through administration. The quails had unlimited access to food and water; after that, food was withheld to avoid the c-Fos immunoreactivity linked to food consumption. Quails were deeply sedated by cardiopuncture with sodium pentobarbital 1 hr after injection when strong c-Fos expression is expected[31]. Ice-cold 0.9% NaCl was then perfused through the carotid artery and 4% paraformaldehyde was added in a pH 7.4 solution of 0.1 M phosphate buffer (PB) with 0.2% picric acid. Brains were removed from the skulls and post-fixed in the same solution for 1 hr. Then, they were blocked and allowed to sink in a series of graded sucrose solutions, 20 and 30% in 0.1 M PB. At 40 low magnification (1 m) coronal slices with appetite-related nuclei were identified using the anatomical descriptions as previously described by Zendehdel *et al.*[29]. The resulting sections were placed in 0.02 M PB saline (PBS) containing 0.1% sodium azide using a cryostat set at -15°C. The arcuate (ARC) data was obtained at 1.60 mm interaural distance, followed by 2.08 mm for the dorsomedial nucleus (DM), ventromedial hypothalamus (VMH), Paraventricular magnocellular (PaMC), two portions of the paraventricular nucleus, lateral hypothalamic region (LH) and parvicellular (PaPC) divisions. Following collection, section processing was completed. The c-Fos immunohistochemistry experiment's methods were previously described by Zhao and Li[32]. For 1 hr, pre-blocked 10% normal goat serum (NGS) and 0.3% Triton X-100 in 0.02 M PBS were used to create the free-floating sections. Sections were treated for 30 min in deionized water containing 1.5% hydrogen peroxide and 50% methanol to stop endogenous peroxidase activity. After being washed three times for 10 min with wash buffer (0.05 and 0.1% NGS), rabbit polyclonal anti-c-Fos (K-25, Santa Cruz, California, USA) was diluted 1:20, 000 and incubated for 48 hrs at a pH of 0.3% in PBS containing 1% NGS, 1% blocking agent (Roche Diagnostics, MA, DE) and 0.3% Triton X-100X.

The main antibody was swapped out for regular rabbit serum for test controls. Following that, sections were incubated for 2 hrs at room temperature with a 1:200 dilution of biotinylated goat anti-rabbit secondary antibody (Vector

Laboratories, California, USA) in PBS containing 1% NGS after being rinsed three times for 10 sec in wash buffer. After a single PBS rinse, Achromatin-biotin horseradish peroxidase complex was used to stain slices at a 1:200 dilution (Vectastain Elite ABC Kit, Vector Laboratories). After 45 sec of reactions using the DAB Substrate Kit for Peroxidase from Vector Laboratories (Vector Laboratories, California, USA), the reactions were mounted using VectaMount and then covered with slips. Anatomical details were verified and each section was photographed using a digital microscope (Olympus DSX1000 digital microscope, Shinjuku-Ku, Tokyo, Japan). Micrographs and overlays containing the boundaries of the individual nuclei were digitally combined and a technician who was unaware of the treatment counted the quantity of c-Fos immunoreactive cells in each nucleus.

Blockage of corticotrophin-releasing factor (CRF) receptor: The experimental birds (fasted for 3 hrs) either got a vehicle alone or 1.0 nmol AM with 3.0 nmol astressin (Peptide Institute, Osaka, Japan) by ICV administration. The astressin acts as a nonselective corticotropin-releasing hormone antagonist which reduces ACTH synthesis studied by Vulliémoz *et al.*[33]. The dose of astressin was modified to Japanese quail based on earlier studies by Saito *et al.*[34] and Tachibana *et al.*[35]. The rate of food intake was estimated at 3 hrs post-injection.

Statistical analysis: Data were tabulated and used for statistical analysis using the R Core Team (2020). Two-way ANOVA was used for all comparisons, followed by Tukey's HSD test, except for locomotor activity over 2 hrs and feed intake over 3 hrs comparison for corticotropin release factor blocks, where one-way ANOVA was used followed by Tukey's HSD, data were plotted and expressed as Mean±Standard error. The differences are considered statistically significant at $p<0.05$.

RESULTS

AM's impact on calorie intake and weight gain: Compared to the groups that were given vehicles, the resulting outcome revealed that ICV administration of AM to Japanese quail resulted in a dose-dependent decrease in feed intake at the different experimental times (2, 4 and 8 hrs) (Fig. 1). This reduction became apparent at 2 hrs, while it became significant ($p\leq0.05$) at 4 and 8 hrs, especially at dose 0.5 and 1 nmol. Similarly, body gain showed a significant ($p\leq0.05$) reduction (Fig. 2). This reduction started at 2 hrs and became more prominent at 8 hrs. Body weight gain at AM-ICV doses of 0.5 and 1 nmol showed a marked reduction compared to the vehicle and 0.01 nmol treated groups. The reduction in body weight gain became more obvious than the decrease in feed intake at 8 hrs.

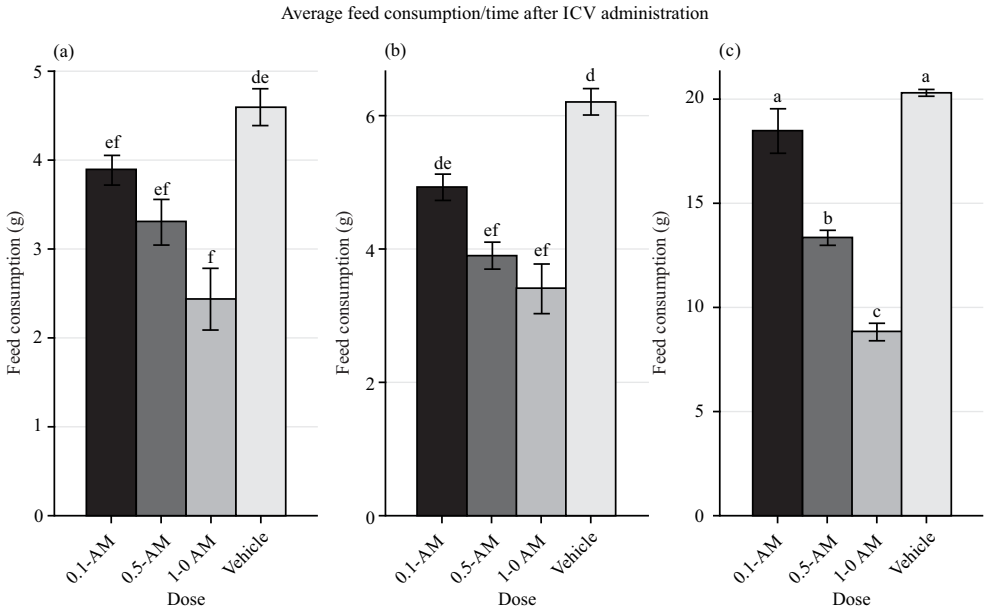

Fig. 1(a-c): Effect of 0.1, 0.5 and 1.0 nmoL of AM-ICV administration on cumulative food intake in Japanese quail, (a) 2 hrs, (b) 4 hrs and (c) 8 hrs

[ef,f,de,a,b,c]Mean values with different superscript letters are significantly different

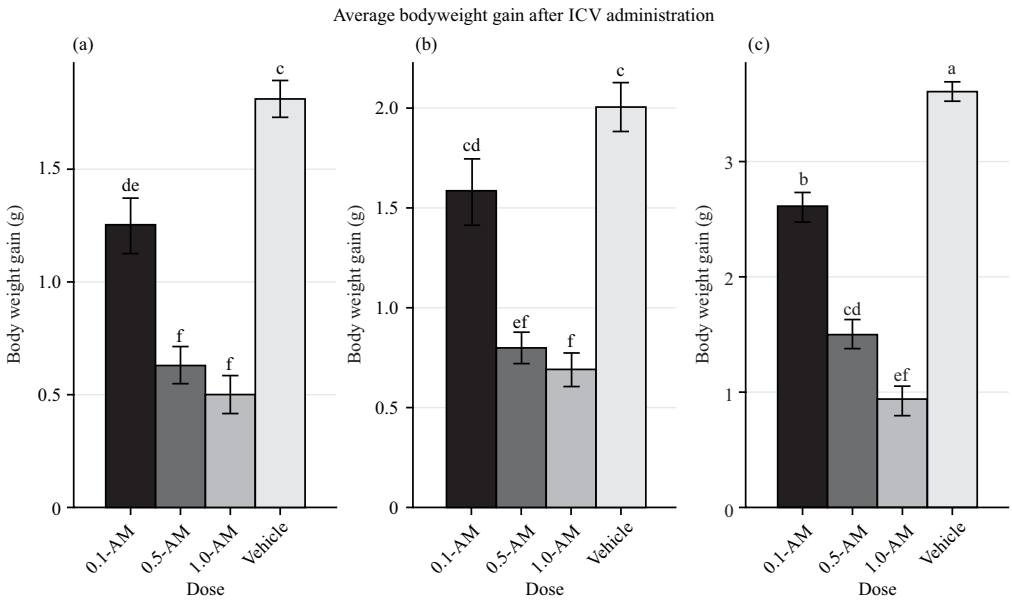

Fig. 2(a-c): Effect of 0.1, 0.5 and 1.0 nmol of AM-ICV administration on body weight gain in Japanese quail, (a) 2 hrs, (b) 4 hrs and (c) 8 hrs

[de,f,cd,ef,a,b,c]Mean values with different superscript letters are significantly different

Effect of AM on water intake: The results provided in (Fig. 3) revealed that ICV of AM in Japanese quails resulted in no significant effect on water intake at any dose (0.1, 0.5 and 1 nmol) or time (2, 4 and 8 hrs).

Effect of AM on body temperature: Soon after the AM-ICV, the bird's body temperature (Fig. 4) increased, especially at 0.5 and 1 nmol doses. This elevation became noticeable at 10 min and continued for 2 hrs. However, this increase was not statistically different from vehicle or AM-ICV 0.1 nmol.

Effect of AM on gross locomotor activity: Gross locomotor activities were examined in different experimental groups and the results showed that gross locomotor activity was increased dose-dependently compared to vehicles in the Japanese quail (Fig. 5).

AM and immunoreactivity: Japanese quails treated with AM-ICV injection had a substantial ($p<0.05$) rise in c-Fos immunoreactivity in the ARC, DM, PaMc, PaPc and VHH compared to control groups (Fig. 6). While LH showed no significance over the vehicle-treated birds.

Blockage of the corticotropin-releasing factor: Japanese quails treated with AM-ICV, astressin, or AM+Astressin consumed significantly less food compared to those treated with vehicles ($p<0.05$) (Fig. 7). However, there were no significant changes between the AM-ICV, astressin and AM+Astressin-treated groups.

DISCUSSION

This study investigated the central AM effect on food and water intake, body temperature, gross locomotor activity and c-Fos reaction in brain tissues in adult Japanese quail as an avian model; the results obtained disclosed significant ($p\leq0.05$) decreases in food intake, body weight gain without affecting water intake and increased the locomotor activities, with variable response in c-Fos reactions in brain tissues. Feeding-related peptide's effects on food consumption of day-old chicks have been examined by ICV administration[36-39].

Yet it is not well established if the 1-day-old chicks can fully control the central regulation of various physiological functions, such as body temperature, feeding and the sleep-wake cycle, so we have used adult Japanese quail that virtually have a constant body temperature and daily food intake and widely used[40,41]. To further eliminate the effects of female reproductive hormones, sex differences and potential interference with the utilized peptides on food consumption and other monitored parameters during this experiment, mature male quails were selected[42].

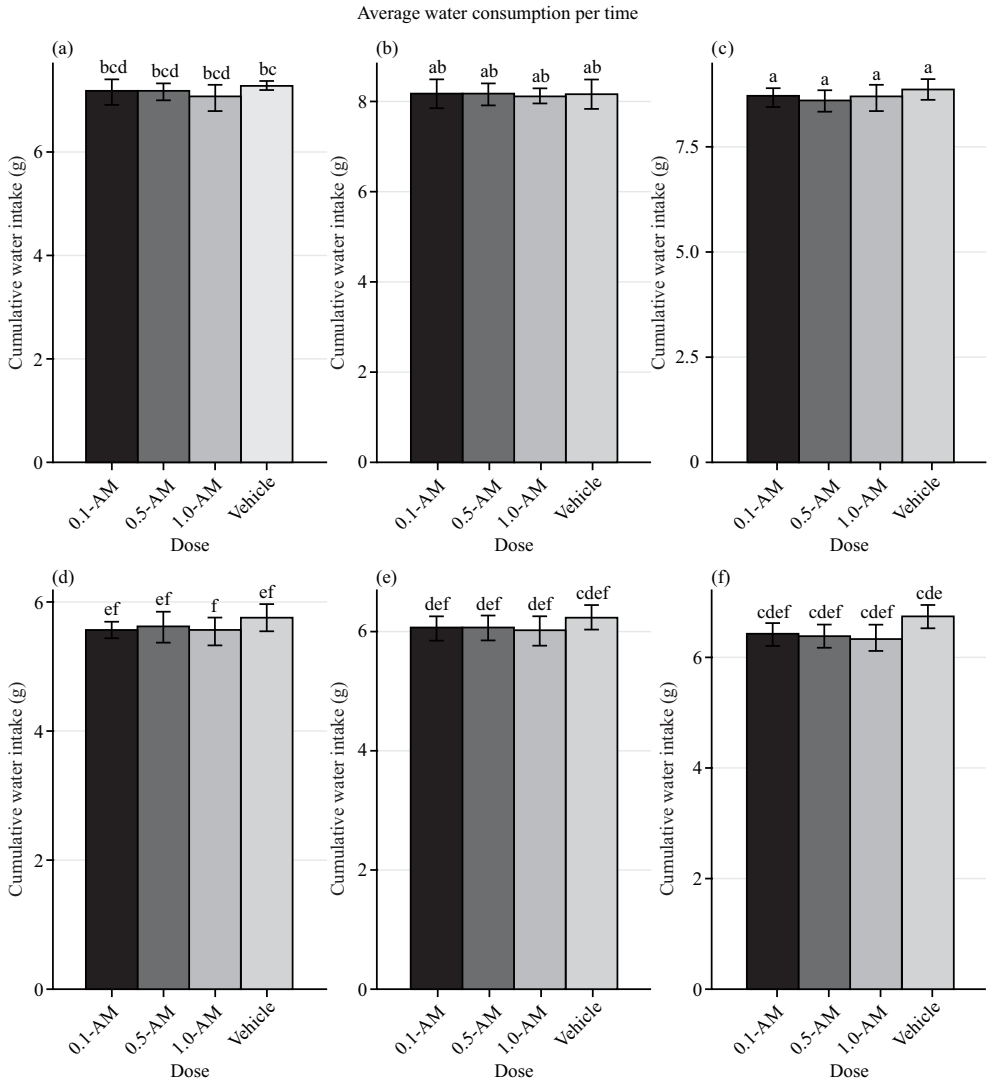

Fig. 3(a-f): Effect of 0.1, 0.5 and 1.0 nmol of AM-ICV administration on cumulative water intake in Japanese quail, (a) 120 min, (b) 150 min, (c) 8 min, (d) 30 min, (e) 60 min and (f) 90 min

[bcd,bc,ab,a,ef,f,def,cdef,cde]Mean values with different superscript letters are significantly different

Neuropeptides are fragments that are synthesized in cells through large inactive precursors of proteins. Many hypothalamic neuropeptides are possibly shared in central feed regulation and energy expenditure. Currently, over 40 neuropeptide precursors are known[43,44].

Lateral ventricles for central injection were reported by Wang *et al.*[45]. In that report, the AM anorexigenic effect in domestic chickens was proved. Moreover, the third ventricle was used and the AM anorexic effect on the Japanese quail was recorded. Similar findings were reported in mammals[19]. Therefore, the administration route does not matter. Previous studies have shown that food intake in rats and goldfish was decreased by central and peripheral injection of amylin, AM2/IMD[19,46,47]. Given that central CGRP and amylin, when administered to chicks, both showed anorexia-inducing effects through CGRP receptors, as previously stated the central AM produces anorexia in rats[24,48].

Data obtained in this study (Fig. 1) matched the earlier study by Wang *et al.*[45], which indicated the negative influence of AM on juvenile chicken and reduced appetite. There are few reports about the effect of AM on food consumption. In general, this work provided reference data on the effect of the exogenous AM in the avian class and is considered a pioneer concerning the Japanese quail, rats experienced anorexia following exogenous AM exposure, while goldfish did not[19,49]. According to a previous study, the anorexigenic

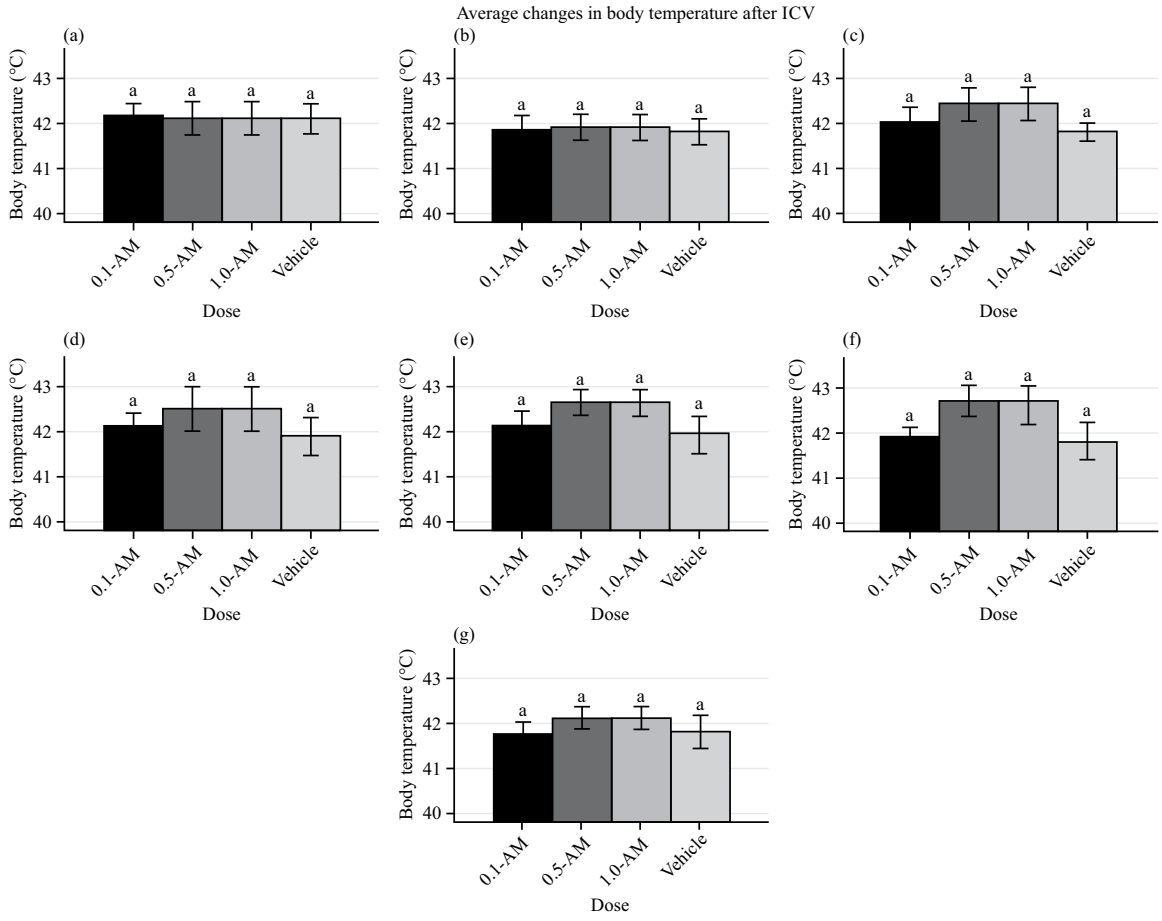

Fig. 4(a-g): Effect of 0.1, 0.5 and 1.0 nmol of AM-ICV administration on body temperature in Japanese quail, (a) 0 min, (b) 5 min, (c) 10 min, (d) 20 min, (e) 40 min, (f) 60 min and (g) 120 min

[a]Mean values with similar superscript letters are not significantly different

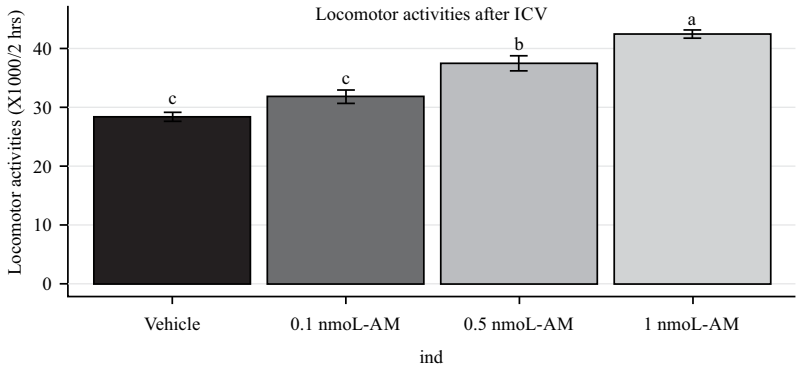

Fig. 5: Effect of 0.1, 0.5 and 1.0 nmol of AM-ICV administration on gross locomotor activity in Japanese quail

[a,b,c]Mean values with different superscript letters are significantly different

AM dosage was between 0.17 and 1.7 nmol at 120 min after administration and between 1.7 and 5 nmol at 30 min after ICV injection[23]. Study by Wang *et al.*[45] reported that the effective dosage threshold was greater in chicks than in rats, although they developed anorexia at a dose 5 times lower than rats did within 120 min of injection[45]. In the current study,

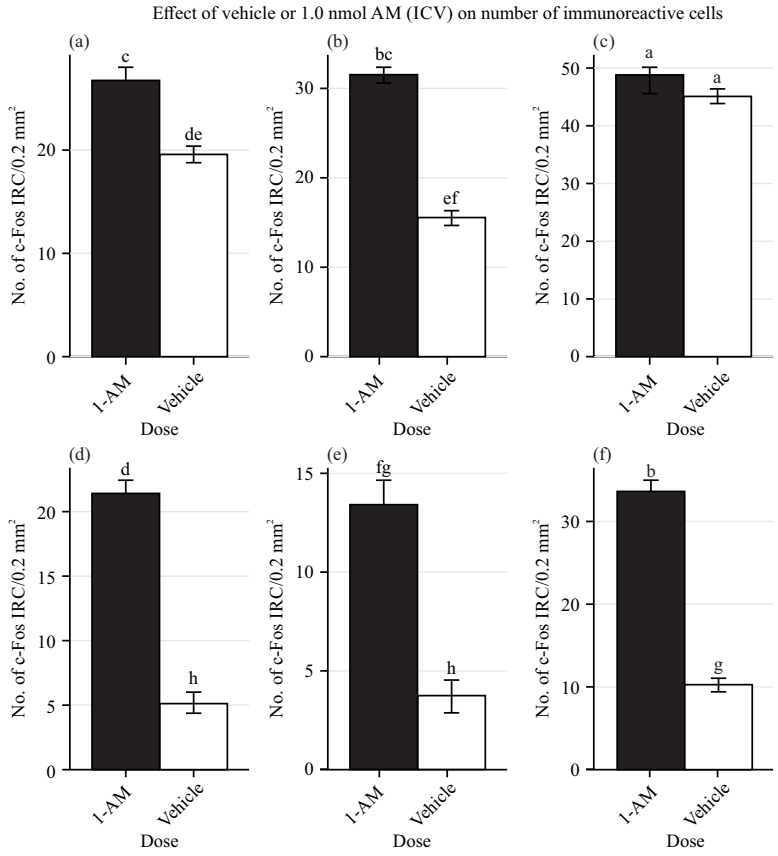

Fig. 6(a-f): Effect vehicle or 1.0 nmol AM-ICV administration on numbers of immunoreactive cells, (a) ARC: Arcuate nucleus, (b) VHM: Ventromedial hypothalamus, (c) DM: Dorsomedial hypothalamus, (d) PaPC: Parvocellular, (e) PaMC: Magnocellular and (f) LH: Lateral hypothalamus

[c,de,bc,ef,a,d,h,fg,b,g]Mean values with different superscript letters are significantly different

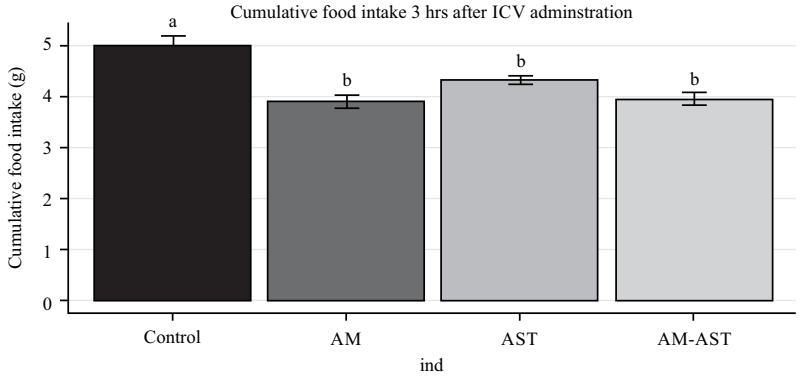

Fig. 7: Cumulative food intake 3 hrs After ICV of vehicle, 1.0 nmol of AM, 3.0 nmol astressin (AST) and AST+AM in Japanese quail

[a,b]Mean values with different superscript letters are significantly different

Japanese quails responded with anorexia at doses of 0.1, 0.5 and 1.0 nmol, which were lower than the doses used in chicks (1.0, 2.0 and 3.0 nmol) and the duration of anorexia in quail was longer (8 hrs after ICV administration) than that reported in chicks or rats. This indicated that AM has a temporary effect on anorexia in chicks; it has a lasting influence on it in rats.

However, in the case of Japanese quails, AM-induced anorexia was longer than that reported in rats or chicks after ICV administration of either a small dose (0.1 and 0.5 nmol) or a large dose (1.0 nmol). However, Wang *et al.*[50] used a dose of 1 and 3 nmol of AM in Japanese quail and disclosed that the anorexic effective dose was 3 nmol. Compared to the animals' ecosystems, the duration of AM-induced anorexia differs in these different experimental birds, favoring a longer or shorter AM duration of induced anorexia[50]. This may be due to the different daytime feeding habits of the rats, chicks and Japanese quail. These variations in animal evolution may be related to variations in receptor function or neuropeptide clearance systems between various animal species. Since the orthologous avian AA sequence and human adrenomedullin peptide have 72% identity and 78% similarity, human adrenomedullin peptide (52 AA) in this work has been chosen in this study. This might significantly impact the threshold and duration of the reaction. In this study, Japanese quails received an ICV injection of AM, which resulted in a substantial ($p<0.05$) decrease in feed intake (Fig. 1).

Previous studies in birds have investigated the effect of AM and other members of the CGRP family (amylin and CGRP) on food intake[21,45,47]. They elucidated that the threshold of anorexia among these 3 CGRP (amylin, CGRP and AM) appear similar. The current results found that the anorexic AM threshold was 0.5-1 nmol in Japanese quail after ICV administration which was similar to that reported in an earlier study but with a longer duration[45]. On the other hand, Wang *et al.*[50] reported that the anorexic AM-ICV dose was 3 nm. An earlier study by Campos *et al.*[51] suggested that anorexic effects mainly occur due to activation of the CGRP receptors and it is possible that AM activated these receptors in rats, chicks and Japanese quails[51].

The effect of AM on water intake showed some controversy. In rats, some reports showed that the AM was hypotensive, showing an anti-dipsogenic effect and associated with reduced water intake[23,52]. In the current study, the dipsogenic effect was not clear and AM-ICV did not cause noticeable variation in water consumption in Japanese quail (Fig. 3). The current study results coincided with earlier studies that stated AM was associated with changes in feed consumption but with little effect on chick's water intake[45,50].

Regarding whether AM plays a role in energy outflow, the effect of AM-ICV administration on Japanese quails' body temperature and gross locomotor activity was investigated. The AM causes transient increases in body temperature (Fig. 4), suggesting its thermogenic effect, which may be physical or chemical and increased gross locomotor activity. These findings imply that AM increased energy consumption. It appears that AM reduced weight gain by consuming less food. It is unclear whether AM stimulates or inhibits lipolysis in avian species; therefore, further research is needed. In this aspect, it was stated that heat loss regulation and thermogenesis were the two chief effects of preserving body temperature[53]. To determine whether the AM can modify the Japanese quail's body temperature, it was injected during the light phase, where the bird was active and the diurnal body temperature was naturally higher. The AM-ICV centrally administrated and produced only transient (at 2 hrs) non-significant ($p\leq0.05$) changes in the bird's body temperature (Fig. 4). The slight and shorter changes in the bird's body temperature might result from AM potentiation to the sympathetic activities, which is usually high. Extra work is required around the exact mechanism by which AM modifies an animal's core temperature and why its duration is concise compared to the change in feed.

The AM affects the hypothalamus's feeding center; the anatomical position of the thermo-regulatory center, which is closely located to the feeding center, may play a role. An early study suggested that both feeding and thermoregulatory centers were linked to each other[54]. Recently, the correlated body temperature changes to feeding energy-thermogenic effects in rats were studied[55]. Increased locomotor activity may contribute to the short changes observed in the bird's core temperature. During current work, results obtained revealed that compared to the vehicle birds' group, there was an AM dose-dependent increase in gross locomotor activity (Fig. 5). Notably, an earlier study proved that AM was required for normal motility and locomotion in mice. Further studies are required to investigate which type of behavior is increased and which is reduced after AM administration in Japanese quail[56].

The hypothalamus plays a central role in birds feeding regulation; therefore, it is necessary to elucidate which hypothalamic nuclei mediate the anorexigenic effect of AM in Japanese quail[57]. Current study results revealed that central AM has increased c-Fos immunoreactivity in some hypothalamic nuclei compared to vehicle-treated birds (Fig. 6). The immunoreactive hypothalamic nuclei may be associated with the anorexigenic effect of AM. Despite discovering other satiety-inducing neuropeptides in this region. Lesion testing showed the VMH as the first satiety-related nucleus, raising issues regarding its role in fullness perception[45,58]. However, a proven role of the VMH in feeding behavior has recently been described by Gaur *et al.*[59]. In terms of hunger control, the DM, a nucleus that has

received less research, also demonstrates high amounts of various appetite variables[60,61]. This includes neuropeptide Y (NPY), cholecystokinin (CCK), CRF and CRF receptors, which were associated with satiety[45].

The PVN is essential for controlling appetite since it expresses anorexigenic and orexigenic substances[62]. The PVN, DM and VMH responded as a result, demonstrating that fullness caused the Japanese quails in our study to reduce their meal intake rather than general effects like malaise. The absence of an anti-dipsogenic impact in this study demonstrated that malaise did not cause a reduction in calorie intake. The AM-ICV has been shown to affect mammalian c-Fos immunoreactivity in earlier investigations. The AM-ICV was found to be connected to PVN activation in rats and in chicks, which agreed with our results[45,63,64]. Thus one might speculate that AM from the PVN in Japanese quail may originate, in addition to projections to specific cells in the DM and VMH. Following AM injection, rats with ablated AP nuclei displayed decreased PVN neuron activity[63].

It has been demonstrated that a variety of neurotransmitters that reduce the food intake in chicks were CRF-dependent[21,22,45,48]. It has been proposed that, in some circumstances, the activation of CGRP receptors may also activate CRF neurons because CRF neurons receive direct input from CGRP nerve terminals[65]. The PVN stimulated the region of CRF genesis in the current study by Mönnikes *et al.*[66]. Current study findings, however, contradicted this because astressin, a CRF receptor antagonist, did not reduce the AM-induced reduction in food consumption. This assures that the anorexic effect produced was not of CRF origin. According to earlier research, central injections of AM had anorexigenic effects in domestic hens via the lateral ventricle[45]. In contrast, the third ventricle of Japanese quails was employed in this study. Additionally, AM had an anorexigenic impact on Japanese quails. The outcomes were identical for both chickens and Japanese quails and both were consistent with those observed in mammals[19,67]. This proved that the injection location was inconsequential in both birds and mammals. Thus, the administration method was different from that of the earlier research done on chickens.

CONCLUSION

We have demonstrated that exogenous human AM-ICV administration has a CRF receptor non-dependent anorexigenic effect, with some behavioral changes including increased locomotor activity, transient change in body temperature, not affecting water intake, alterations in the chemistry of the hypothalamus, as well as the activation of the VMH, DM and PaPC in Japanese quail.

SIGNIFICANCE STATEMENT

This study examined the anorexic and hypotensive effects of adrenomedullin with different doses to ascertain its mechanism of action. Albeit, adrenomedullin effects have been observed earlier in several species, including Japanese quail, yet tried to investigate its effect by measuring extensive and more reliable parameters such as locomotor activity, body temperature, immunoreactivity in the dorsomedial hypothalamus (DM), ventromedial hypothalamus (VMH), paraventricular magnocellular (PaMC), paraventricular parvocellular (PaPc) and arcuate nucleus (ARC), lateral hypothalamus (LH) which signifies the importance of this study and underlines it's a novelty. Additionally, it is also attempted to rule out its effects as corticotropin-releasing hormone antagonists using astressin. The findings are interesting and make this study important from a scientific point of view.

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