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ORIGINAL PAPER

Ghrelin-induced hypophagia is mediated by the β_2 adrenergic receptor in chicken

Morteza Zendehdel · Shahin Hassanpour

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Abstract The purpose of this study was to examine the effects of intracerebroventricular injection of metoprolol (a β_1 adrenergic receptor antagonist), ICI 118,551 (a β_2 adrenergic receptor antagonist), and SR 59230R (a β_3 adrenergic receptor antagonist) on ghrelin-induced food and water intake by 3-h food-deprived (FD_3) cockerels. The chickens were randomly allocated to 4 treatment groups with 8 replicates in each group. A cannula was surgically implanted into the lateral ventricle of the brain. In experiment 1, chickens received the β_1 adrenergic receptor antagonist (24 nmol) before injection of the ghrelin (0.6 nmol). In experiment 2, chickens received the β_2 adrenergic receptor antagonist (5 nmol) before injection of the ghrelin (0.6 nmol). In experiment 3, birds were injected with ghrelin (0.6 nmol) after the β_3 adrenergic receptor antagonist (20 nmol). Cumulative food and water intake were recorded 3-h post injection and analyzed by two-way analysis of variance. According to the results, ghrelin injection reduced food and water intake by broiler cockerels ($p \leq 0.05$). The effect of ghrelin on food intake was significantly attenuated by pretreatment with the β_2 receptor antagonist ($p \leq 0.05$). Furthermore, the β_2 receptor antagonist had no effect on water intake induced by ghrelin. Also, pretreatment with the β_1 and β_3 receptors antagonists had no effect on ghrelin-induced food and water intake. These results suggest that the effect of ghrelin on cumulative food intake by cockerels is mediated via β_2 adrenergic receptors.

Keywords Ghrelin · β Adrenergic receptor · Food and water intake · Chicken

M. Zendehdel (✉)
Department of Physiology, Faculty of Veterinary Medicine, University of Tehran, 14155-6453 Tehran, Iran
e-mail: zendedel@ut.ac.ir

S. Hassanpour
Section of Physiology, Department of Basic Sciences, Faculty of Veterinary Medicine, Science and Research Branch, Islamic Azad University, Tehran, Iran

Introduction

Broiler cockerels, because of their high growth rate and production, among the most beneficial domestic animals. There is evidence that food-intake regulatory mechanisms are different for layer and broiler chickens, because of genetic differences [1]. It is well known that the recently discovered compound ghrelin stimulates food intake in mammals [2, 3]. Although accumulating evidence implies ghrelin is important in mammals, there is little evidence of its effect on other vertebrates. It seems avian ghrelin (a 26-amino-acid peptide) is produced by the brain, lung, and spleen, with the highest expression in the proventriculus [4]. Abdominal fat, small intestine, and breast muscle [5] provoke growth hormone (GH) release via GH secretagogue receptors (GHS-R) [2, 3]. Three subtypes of the ghrelin receptor have been identified in chicken: GHS-R1a, GHS-R1av (homologous with mammalian GHS-R1b), and GHS-R1tv [6]. Recent research has demonstrated that exogenous administration (peripheral or central) of ghrelin increases food intake by laboratory animals [7–9] whereas intracerebroventricular (ICV) injection inhibits food intake by broiler chickens [1, 10–12] and adult Japanese quail [3]. In mammals, there are two sites in the hypothalamic arcuate nucleus (ARC) which have crucial effects on food intake by producing neuropeptide Y (NPY) and agouti-related protein (AgRP). Previous research has suggested AgRP increases cumulative food intake by layer hens but

not by broiler cockerels [13, 14] and that the suppressive effect of ghrelin on chickens might be mediated by corticotropin-releasing factor (CRF) [4]. Although mechanisms of interaction with food intake by broiler chickens is unclear, it has been suggested food intake by avian species is mediated by the ARC [1, 4, 12, 15–18]. Although much progress has been made in identifying mediatory effects of ghrelin on feeding behavior in different parts of the central nervous system (CNS), less research has been conducted to verify the effects of ghrelin on drinking by chickens. Brain areas associated with body fluid homeostasis in response to ghrelin have recently been identified [19]. Kozaka et al. [20] have demonstrated that water intake by seawater-acclimated eels was reduced by intracranial injection of ghrelin.

Effects of β adrenergic receptors on body metabolism have been detected, and a mediatory effect of β adrenergic on appetite is possible. It is well-documented that intravenous (IV) injection of isoproterenol (a nonselective β adrenergic receptor agonist) reduces food intake by adult rats [21], and that ICV administration of a β adrenergic receptor antagonist increases food and water intake by rats. It can be concluded that the adrenergic system has mediatory effect on the control of food and water intake by rats [22]. The distribution of adrenergic receptors differs in different parts of the avian brain. It is known that α_2 adrenergic receptors increase food intake by poultry whereas β adrenergic receptors reduce food and water intake [23]. In contrast, intraperitoneal (IP) administration of BRL37344 (a β_3 adrenergic receptor agonist) to rats resulted in a significant decrease in food intake [24]. We have previously reported an interaction between ghrelin and glutamatergic and serotonergic systems in broiler chickens [12, 25], and it has been reported that central norepinephrine reduces appetite in mammals [26] and chicken [23]. Similarly, research has demonstrated that noradrenergic neurons may regulate water intake via other neural systems [27]. For example, norepinephrine level is increased by injection of ghrelin into the ARC [10, 15]. It thus seems that central norepinephrine release is mediated via ghrelin [10]. On the basis of these findings and considering the same effects of ghrelin and β adrenergic receptors on the feeding behavior of birds, we hypothesized that the adrenergic system possibly mediates ghrelin signaling in the hypothalamus of 3-h food-deprived (FD_3) birds.

Materials and methods

Animals

Day-old ROSS 308 broiler chickens (Eshragh, Iran) were weighed and randomly located in 4 treatment groups with 8 replicates in each group (average live body weight (LBW) in each group 920 ± 50 g). Two experimental diets were offered during the study, a starter diet containing 20 % crude protein (CP) and 2900 kcal/kg metabolizable energy (ME), and 19 % CP and 2950 kcal/kg ME as a grower diet. Chickens were reared in heated batteries until 2 weeks of age with continuous lighting, at 22 ± 1 °C and with 50 ± 2 % humidity [28]. Animal handling and experimental procedures were performed in accordance with the guide for the care and use of laboratory animals by the National Institutes of Health (USA) and the current laws of the Iranian government.

Experimental drugs

Metoprolol (a β_1 adrenergic receptor antagonist), ICI 118,551 (a β_2 adrenergic receptor antagonist), SR 59230R (a β_3 adrenergic receptor antagonist), and acylated ghrelin were purchased from Tocris Bioscience (UK). All solutions were prepared in pyrogen-free 0.9 % NaCl solution (saline) that served as control.

Surgical procedures

At 3 weeks of age, for implantation of ICV cannulas, the broilers (approximately LBW 750 g) were anesthetized by intramuscular (IM) injection of 1 mg/kg body weight xylazin and 30 mg/kg body weight ketamine [29]. A stainless steel (23-gauge thin-walled) guide cannula (Razipakhsh, Iran) was stereotaxically implanted in the right lateral ventricle by use of a technique reported elsewhere [30]. The coordinates were 6.7 mm anterior of the bregma, 0.7 mm lateral of the midline, and horizontally 3.5–4 mm below the dura mater with the head oriented as described by Denbow et al. [30]. Three stainless-steel screws and acrylic dental cement (Pars Acryl, Iran) were used to immobilize and protect the guide cannula. The screws were placed in the calvarias and the cement was then applied to the screws and the guide cannula. In non-injection periods between experiments, orthodontic no. 014 wire (American Orthodontics, USA) trimmed to the precise length of the guide cannula and inserted into the cannula. Lincospectin (Razak Company, Iran) was applied to incisions to prevent possible infection.

Experimental procedures

To determine possible effects of β_1, β_2, and β_3 adrenergic receptor antagonists on ghrelin-induced feeding and drinking responses of chickens, metoprolol, ICI 118,551, and SR 59230R were administered by use of a thin-walled (29-gauge) stainless steel injecting cannula (Razipakhsh, Iran) which extended 1.0 mm beyond the end of the guide

Table 1 Treatments procedures in experiments 1–3

Treatment group	First injection (5 μl)	Second injection (5 μl)
Exp. 1		
A	S*	S*
B	S*	Ghrelin (0.6 nmol)
C	Metoprolol (80 μg)	S*
D	Metoprolol (80 μg)	Ghrelin (0.6 nmol)
Exp. 2		
A	S*	S*
B	S*	Ghrelin (0.6 nmol)
C	ICI 118,551 (5 nmol)	S*
D	ICI 118,551 (5 nmol)	Ghrelin (0.6 nmol)
Exp. 3		
A	S*	S*
B	S*	Ghrelin (0.6 nmol)
C	SR 59230R (20 nmol)	S*
D	SR 59230R (20 nmol)	Ghrelin (0.6 nmol)

The time between injections was 15 min

*S** saline, 0.9 % NaCl, *metoprolol* β_1 adrenergic receptor antagonist, *ICI 118,551* β_2 adrenergic receptor antagonist, *SR 59230R* β_3 adrenergic receptor antagonist

cannula. The injecting cannula was attached to a 10-μl Hamilton syringe by use of a piece of polyethylene-20 tubing 60 cm long (Parsian Tube, Iran). Solutions were injected over a period of 60 s, during which time the solution was allowed to diffuse from the tip of the cannula into the ventricle. Experiments were performed from 9:00 a.m. until 1:00 p.m. Before injection, broiler chickens were taken from their individual cages and restrained by hand; after the injections they were immediately returned to the cages. To adapt the chickens to the injection process and lessen palpation stress, for a period of 5 days before the experiments the cockerels were moved and mock-injected daily. Three hours before initiation of the injections, the animals were deprived of food (FD_3). Immediately after the injections the animals received fresh food and water. Three experiments were designed, each with four treatment groups: Control (A), B, C and D groups ($n = 8$ per group). In experiment 1, chickens received the β_1 adrenergic receptor antagonist (24 nmol) before injection of ghrelin (0.6 nmol). In experiment 2, chickens received the β_2 adrenergic receptor antagonist (5 nmol) before injection of the ghrelin (0.6 nmol). In experiment 3, birds were injected with the ghrelin (0.6 nmol) after β_3 adrenergic receptor antagonist (20 nmol). Cumulative food intake (g) and water intake (ml) were then measured automatically 15, 30, 60, 120, and 180 min post injections. In a similar manner to the treatment groups, all control groups received two injections of 5 μl saline, at 15 min intervals. Placement of the guide cannula in the ventricle was verified by the presence of cerebrospinal fluid and by ICV injection of methylene blue followed by slicing the frozen brain tissue at the end of each experiment. Details of the injection procedures are given in Table 1. The time between two injections was 15 min. In this study, doses of metoprolol, ICI 118,551, SR 59230R, and ghrelin doses were selected on the basis of preliminary and previous studies [12, 23, 25].

Measurement of food and water consumption

The broilers were returned to individual, automated metabolic cages (Altromin, Germany) immediately after injection. Cumulative food consumption (g) was automatically recorded 15, 30, 60, 120, and 180 min after injection. Any food spillage was collected and weighed [3]. Water consumption (ml) was determined automatically, by use of a graduated water container, at the same times.

Statistical analysis

Cumulative food and water intake was analyzed by two-way analysis of variance (ANOVA), by use of SPSS 16.0 for Windows; results are presented as mean ± SEM. For treatments found to have an effect according to the ANOVA, mean values were compared by use of post hoc Bonferroni and Dunnett tests. p values <0.05 were considered to indicate significant differences between treatments.

Results

The effects of β_1, β_2, and β_3 adrenergic receptor antagonists on ghrelin-induced food and water intake by broiler cockerels are presented in Figs. 1, 2, 3, 4, 5 and 6. As is apparent from Fig. 1, a single ICV injection of metoprolol (β_1 adrenergic receptor antagonist, 24 nmol) had no significant effect on food intake compared with the control group ($p \geq 0.05$). A significant decrease in food intake was detected on injection of ghrelin (0.6 nmol) only ($p \leq 0.05$; $F(3, 25) = 11.85$). Pre-injection of metoprolol did not alter the reduction of food intake caused by ghrelin (Fig. 1) ($p \geq 0.05$).

ICV injection of ICI 118,551 (β_2 adrenergic receptor antagonist) (5 nmol) had no significant effect on food intake compared with the control group ($p \geq 0.05$). A significant ($p \leq 0.05$) decrease in food intake by FD_3 cockerels was detected after injection of ghrelin (0.6 nmol). Pre-injection of ICI 118,551 significantly attenuated the food intake reduction caused by ghrelin (Fig. 2) ($p \leq 0.05$; $F(3, 25) = 14.09$). These results imply

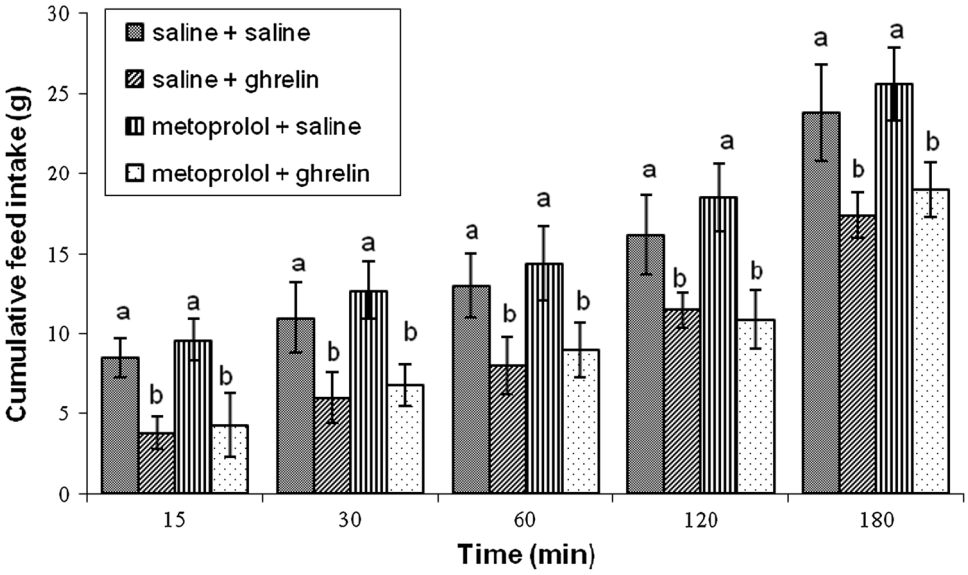

Fig. 1 Effect of ICV injection of metoprolol (24 nmol) (β_1 adrenergic receptor antagonist) followed by ghrelin (0.6 nmol) on cumulative food intake (g) by 3-h food-deprived broiler cockerels. Data are mean ± SEM. There are significant differences between columns with different letters (a or b) in the same group ($p \leq 0.05$)

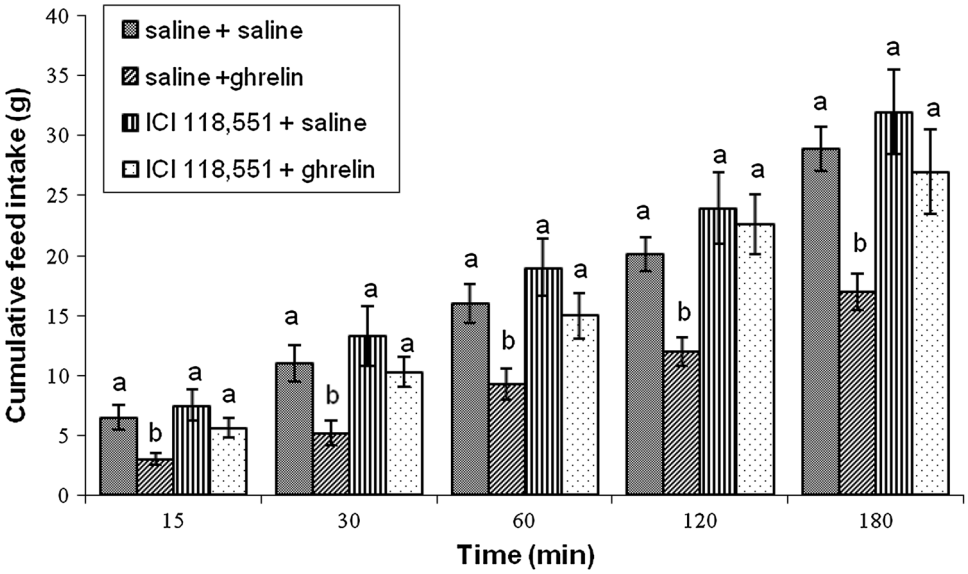

Fig. 2 Effect of ICV injection of ICI 118,551 (5 nmol) (β_2 adrenergic receptor antagonist) followed by ghrelin (0.6 nmol) on cumulative food intake (g) by 3-h food-deprived broiler cockerels. Data are mean ± SEM. There are significant differences between columns with different letters (a or b) in the same group ($p \leq 0.05$)

an interaction exists between the effects of ghrelin and β_2 adrenergic receptors on food intake by FD_3 chickens.

ICV injection of SR 59230R (20 nmol) had no significant effect on ghrelin-induced food intake compared with the control group ($p \geq 0.05$), and pre-injection of β_3 adrenergic receptor antagonist did not attenuate ghrelin-induced hypophagia 15, 30, 60, 120, and 180 min after the second injection ($p \leq 0.05$). Also, there was no significant difference between food intake after SR 59230R + ghrelin compared with ghrelin alone (Fig. 3) ($p \geq 0.05$). We thus hypothesize that the suppressive effect of ghrelin on cumulative food intake is not mediated by β_3 adrenergic receptors.

The effects of ICV injection of metoprolol (24 nmol) and ICI 118,551 (5 nmol) followed by ghrelin (0.6 nmol) on cumulative water intake by broiler cockerels are shown in Figs. 4 and 5. ICV injection of β_1 and β_2 adrenergic receptor antagonists had no significant effect on water intake by FD_3 chickens compared with the control group ($p \geq 0.05$). ICV injection of ghrelin reduced water intake 60 min post-injection ($p \leq 0.05$; $F(3, 25) = 15.48$), and pre-injection of metoprolol and ICI 118,551 did not attenuate the suppressive effect of ghrelin on water intake ($p \leq 0.05$). This implies the effect of ghrelin on water intake is not mediated by β_1 and β_2 adrenergic receptors.

ICV injection of β_3 adrenergic receptor antagonist (20 nmol) followed by ghrelin (0.6 nmol). A single injection of SR 59230R did not alter water intake ($p \geq 0.05$) but significant reduction of water intake was observed 60 min after injection of ghrelin ($p \leq 0.05$). Pre-injection of SR

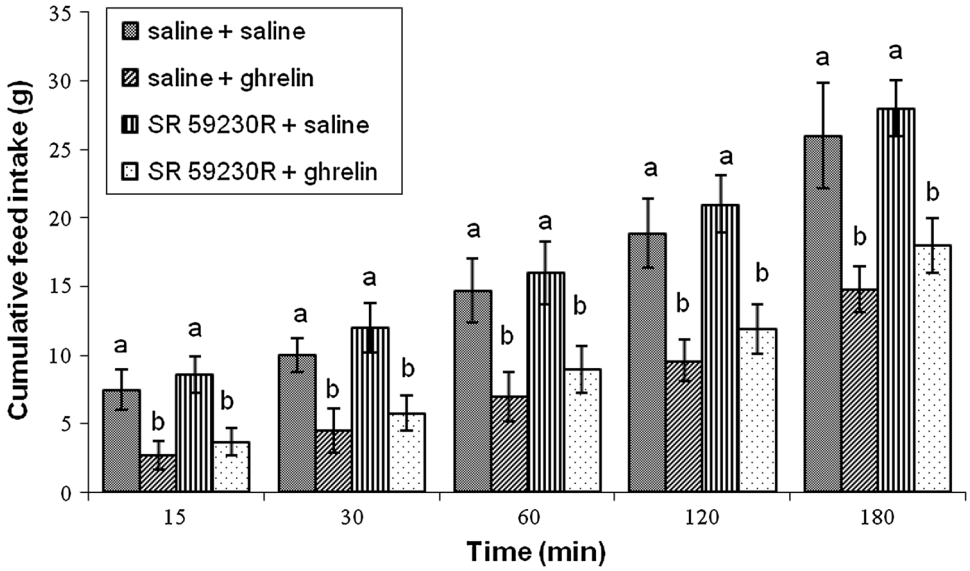

Fig. 3 Effect of ICV injection of SR 59230R (20 nmol) (β_3 adrenergic receptor antagonist) followed by ghrelin (0.6 nmol) on cumulative food intake (g) by 3-h food-deprived broiler cockerels. Data are mean ± SEM. There are significant differences between columns with different letters (a or b) in the same group ($p \leq 0.05$)

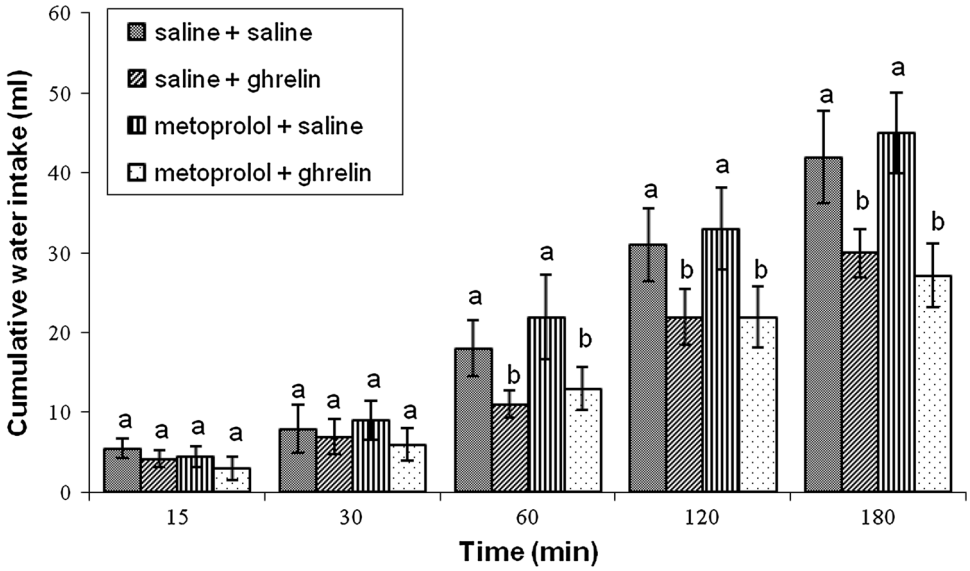

Fig. 4 Effect of ICV injection of metoprolol (24 nmol) (β_1 adrenergic receptor antagonist) followed by ghrelin (0.6 nmol) on cumulative water intake (g) by 3-h food-deprived broiler cockerels. Data are mean ± SEM. There are significant differences between columns with different letters (a or b) in the same group ($p \leq 0.05$)

59230R did not prevent the ghrelin-induced reduction of water intake (Fig. 6) ($p \geq 0.05$). This implies the effect of ghrelin on water intake is not mediated by β_3 adrenergic receptors.

Discussion

Although ghrelin was discovered 15 years ago, its different physiological effects on different types of animal have not been fully investigated, and interaction of ghrelin with other neurotransmitters has not yet been fully clarified [3]. This study was designed to determine the regulatory effect of β adrenergic receptors on ghrelin-induced food and water intake by broilers. Our results indicate ghrelin significantly reduced cumulative food and water intake 3 h after injection of FD_3 cockerels. The effect of ghrelin on food intake by chickens is different from that for mammals. There is evidence of differences between the food-intake regulatory mechanisms of layer and broiler chickens, because of genetic differences between the breeds [31]. For instance, comparative physiological studies on meat-type (broilers) and layer-type (hens) chickens revealed that broilers have higher feed consumption, basal metabolic rate, and energy expenditure, possibly because of a genetically altered mechanism of food-intake control [13, 14]. For layer chickens, but not broilers, AgRP increases food intake. In contrast, ICV injection of NPY increases food intake by both broiler and layer chickens and by mammals [13]. Therefore, although the CNS is involved in

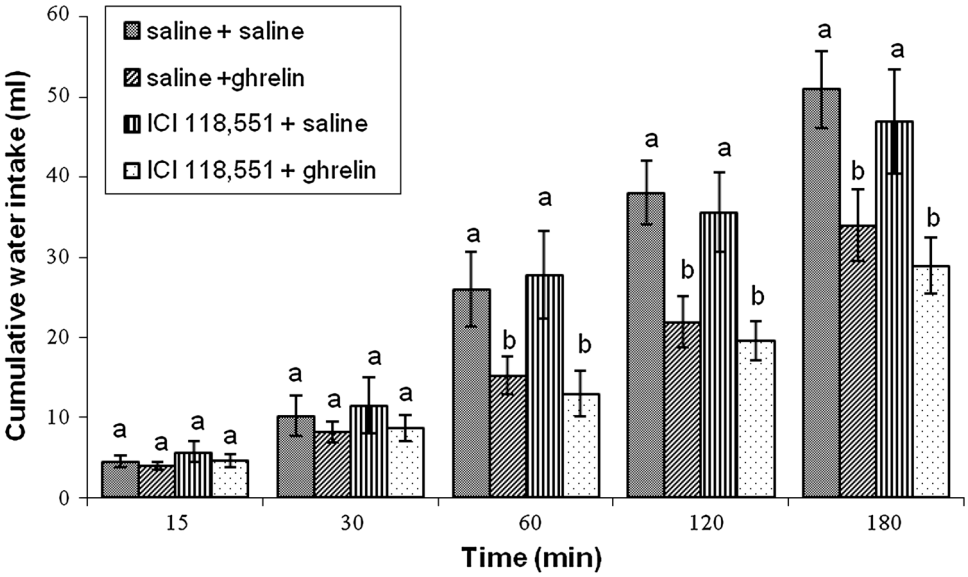

Fig. 5 Effect of ICV injection of ICI 118,551 (5 nmol) (β_2 adrenergic receptor antagonists) followed by ghrelin (0.6 nmol) on cumulative water intake (ml) by 3-h food-deprived broiler cockerels. Data are mean ± SEM. There are significant differences between columns with different letters (a or b) in the same group ($p \leq 0.05$)

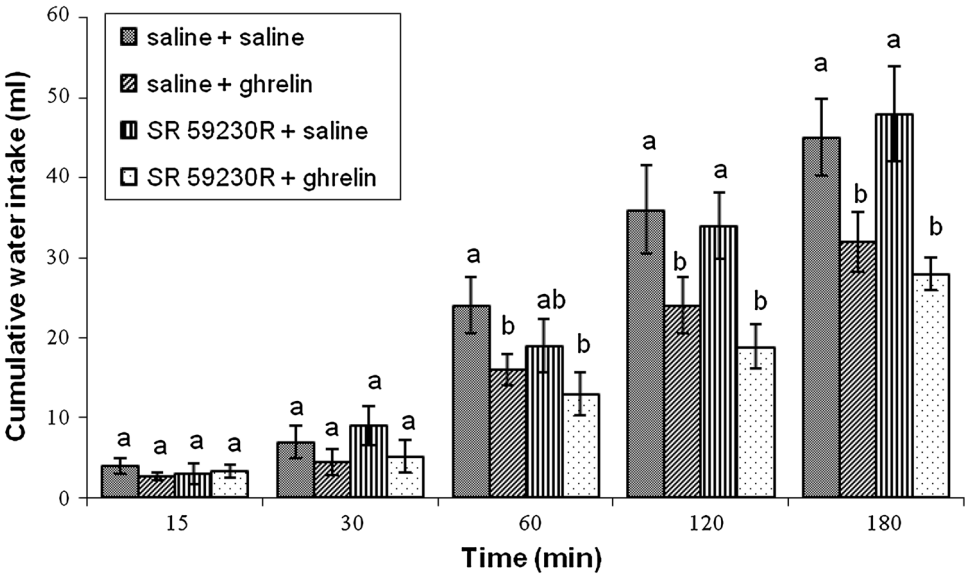

Fig. 6 Effect of ICV injection of SR 59230R (20 nmol) (β_3 adrenergic receptor antagonist) followed by ghrelin (0.6 nmol) on cumulative water intake (ml) by 3-h food-deprived broiler cockerels. Data are mean ± SEM. There are significant differences between columns with different letters (a or b) in the same group ($p \leq 0.05$)

appetite regulation among avian species, other sites are also involved. Genetic selection for meat or egg production has altered chicken brain neurological pathways associated with food intake [4]. On the basis of extensive investigations of mammalian species, it is clear that, despite the differences, food intake may be regulated by similar pathways in birds and mammals. It is, for example, well-documented that administration of β adrenergic receptor agonists reduces food intake by rats [21, 22, 24] and poultry [23]. Despite the similar central regulation of food intake in avian and mammalian species, there are controversial differences between them [2]. Previous studies have revealed that ghrelin has different effects on food intake by different animals. It stimulates feeding by rats, sheep, goldfish, and humans but has negative effects among juvenile fish and quail [32]. Injection of ghrelin causes an increase in food intake by mammals, by activating NPY and AgRP neurons [1–3], but the opposite effect on food intake by chickens compared with rats and humans. For chickens, systemic and ICV injection of ghrelin or other growth hormone secretagogues, for example GHRP-2, strongly inhibits food intake in a dose-dependent manner [1, 11]. Molecular studies have revealed that the avian preproghrelin gene affects five exons and four introns, which is analogous with the human gene, with the exception of the first exon [33]. ICV injection of ghrelin inhibits food intake by Japanese quails and chickens. So, it seems that the different effects of ghrelin on avian and mammal food intake regulation is not related solely to the molecular structure of the ghrelin, and that other mechanisms may be

included, for example different neurological pathways. The mechanism underlying this phenomenon is unclear [3]. Furthermore, ghrelin has different effects on broilers and layers. ICV injection of ghrelin always inhibits food intake by broilers but has no effects on layers [32]. Administration of ghrelin to adult Japanese quail at a low and high doses increased and reduced food intake, respectively, [34]. Such discrepancies may be because of species differences and the site of the brain affected. Saito et al. [10, 11] reported that ICV injection of ghrelin reduces avian food intake and increases serum corticostrone levels. Likewise, a dose-dependent decrease of food intake was observed after ICV injection of ghrelin (0.3, 1.1, 4.3, and 6.2 nmol) to 8-week-old broilers [35]. Consistent with this hypothesis, a significant decrease in food intake by Japanese quail was observed after ICV injection of 1 nmol ghrelin [3]. These data suggest that ghrelin causes reduced food intake by birds. Bungo et al., [26] reported that hypothalamic injection of noradrenaline reduced food intake by mammals. For broilers, paraventricular and ventromedial injection of norepinephrine amplified food intake whereas injection into the tractus occipitomesencephalicus, reticularis superior, and pars dorsalis lessened food intake [36].

A similar finding suggests adrenergic synapses are involved in regulation of food intake by the CNS [26]. Suppression of water intake by broilers [23] and rats [21, 22, 24] after ICV injection of a β adrenergic receptor agonist has been reported. Ferrari et al. [37] theorized that lateral hypothalamic adrenergic receptors have dual effects (stimulatory and/or inhibitory) on cumulative food intake regulation. Previous research has demonstrated that water intake regulatory nucleuses are mediated via dopaminergic neurons and that dopamine may control water consumption via noradrenergic receptors [27]. Conversely, it has been reported that the effect of isoproterenol on water consumption is mediated by the renin-angiotensin system [23, 38]. In our study, pre-injection of a β_2 adrenergic receptor antagonist did not diminish the suppression of water intake by ghrelin, a result similar to that in a study of seawater eels by Kozaka et al. [20]. The new evidence from our study may identify a possible effect of ghrelin on body fluid homeostasis in birds. Hashimoto et al. [39] revealed that ICV injection of ghrelin (1 nmol/kg) suppresses water intake and increases food intake by rats, but that water consumption increases 180 min post-injection. They reported that these increases may result from feeding-associated drinking, because food intake increased markedly 60 min post-injection. It seems the direct mechanism of ghrelin-induced attenuation of water intake is more complicated in chickens. It has been suggested that inhibition of drinking behavior by ghrelin is mediated via osmotic or hypovolemic thirst receptor signals [19], i.e. ghrelin reduces water intake by an antidiuretic effect. It amplifies plasma arginine vasopressin (AVP) levels in rats, although AVP itself has little effect on thirst of rats and dogs [39]. It is important to note that findings presumably depend on the strain, species, and age of broilers. In this study, the inhibitory effect of ghrelin on food intake was significantly attenuated by pretreatment with β_2 adrenergic receptor antagonist. Ghrelin is an androgenic peptide, mostly expressed in the ARC, which modulates animals' appetite [40]. Appetite stimulatory and inhibitory neurons are both present in the ARC. Ninety-four and 8 % of ghrelin receptors in the rat brain express NPY and pro-opiomelanocortin (POMC), respectively; of these, the NPY has a stimulatory effect whereas POMC inhibits food intake [41]. In general, release of some neurotransmitters e.g. norepinephrine, is mediated by ghrelin [42]. It is suggested that ghrelin signaling travels via the vagus nerve to the nucleus tractus solitarious (NTS) and up-regulates noradrenaline release via the α_1 and β_2 receptors, which increases food intake by rats. It is possible ghrelin activates NPY/AgRP neurons in the ARC via the noradrenergic system. It has been reported that peripheral injection of ghrelin activates neurons located in the ARC of rats. It seems ghrelin-induced Fos-positive neurons in the NTS and ARC express NPY in rats, leading to orexigenic effects [43]. These findings suggest the ARC is of crucial importance in regulating peripheral ghrelin signals. However the direct mechanism of ghrelin-induced hypophagia in poultry is still unclear. According to Saito et al. [10], ghrelin-induced hypophagia is mediated by CRF in cockerels. The effect of ghrelin on corticostrone release is more distinct in chickens than in rats and humans [34]. Furthermore, previous results implied that IV injection of ghrelin increases norepinephrine levels in the ARC [15, 42], and a significant increase in dopamine β-hydroxylase expression in the NTS was induced by IV injection of ghrelin. A few reports suggest that noradrenergic fibers penetrate the NTS. The presence of these fibers is essential for regulation of food intake in rats. Thus, any surgical cut in the ARC and lateral brain inter pathway neurons will obscure the restrictive effects of ghrelin on food intake in rats [15]. ICV injection of ghrelin increases dopamine β hydroxylase mRNA in the NTS and increases noradrenalin levels in the ARC, thereby stimulating feeding response via α_1 and β_2 adrenergic receptors in rats. Ghrelin-induced feeding in rats is also attenuated by α_1 or β_2 antagonists. Noradrenalin excites approximately half of the neurons in the ARC, probably because of a direct postsynaptic response via α_1 or β receptors. Peripherally administered ghrelin may activate NPY/AgRP neurons in the ARC through the noradrenalin system [43]. In chicken, β_1 and β_3 receptor antagonists had no effect on ghrelin-induced food and water intake. This implies the β_2 receptor antagonist had no effect on water intake induced by ghrelin. These results suggest that the effect of ghrelin on

cumulative food intake by cockerels is mediated via β_2 adrenergic receptors. Differences among avian and other species of the effect on ghrelin-induced hypophagia must be because another mechanism is involved. As far as we are aware, no previous study has investigated involvement of the central adrenergic system in ghrelin induced-hypophagia among birds. Ghrelin also stimulates release of a variety of hormones and might, thus, lead to antidipsogenic effects indirectly via a hormone, including atrial natriuretic peptide [38] or angiotensin II in rats [44]. Consistent with several lines of evidence, and on the basis of our knowledge, it seems an interaction between ghrelin and the noradrenergic system affects food intake but not water consumption by broilers. It is possible the suppressive effect of ghrelin on food intake is mediated by β_2 adrenergic receptors.

To conclude, we recommend further research to clarify any direct interaction of ghrelin with other neurotransmitters in the feeding behavior of poultry.

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Conflict of interest The authors report no conflicts of interest.

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