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ORIGINAL PAPER

Intralateral hypothalamic area injection of isoproterenol and propranolol affects food and water intake in broilers

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Abstract The role played by adrenergic system in water intake, especially food intake, has long been known in mammals. In avian species, there have been many experiments exploring the effects of the adrenergic system in different sites in the central nervous system in meat- and egg-type poultry. This study was designed to examine the possible effects of intralateral hypothalamic area (ILHy) microinjections of a beta-adrenergic agonist, isoproterenol, and a beta-adrenoceptor blocker, propranolol, on food and water intake in 3-h food-deprived and 3-h water-deprived broiler cockerels. Our findings suggest that the β-adrenergic system directly affects food especially water intake in broilers. Although isoproterenol significantly ($P \leq 0.05$) decreased food intake for the first 15 min, it reduced food intake during the experiment. Isoproterenol reduced water intake significantly ($P \leq 0.05$), which was abolished by pretreatment with propranolol. It is proposed that β-adrenoceptors in LHy play a direct and indirect role in the regulation of food especially water intake in broiler cockerels.

Keywords Intralateral hypothalamic area (ILHy) · Isoproterenol · Propranolol · Food intake · Water intake

Introduction

For many years a dual-center hypothesis was served to explain the neural regulation of food intake in mammals (Stellar 1954). It was based on the work of Hetherington and Ranson (1940), who demonstrated in rats that bilateral lesions of ventromedial hypothalamic nuclei (VMN) resulted in increased food intake and marked obesity. Further research involving bilateral lesions of the lateral hypothalamic area (LHy) resulted in an opposite effect, complete absence of feeding and drinking, and a marked loss in body weight (Anand and Brobeck 1951). As the knowledge of the neuronal network and their ramifications has expanded, the view of functional "centers" has been replaced by that of discrete neuronal populations, expressing specific neurotransmitters that mediate particular effects on food intake and/or energy expenditure and that are regulated by specific signals of nutritional state (Williams et al. 2001).

A primary central nervous site for control of energy balance is the hypothalamus. This region is also intimately associated with the regulation of basic functions such as thirst, reproduction, temperature, hormonal balances, and biological rhythms. The hypothalamus consists of more than 40 histologically distinct nuclei and areas, which can be further divided into different subnuclei. Hypothalamic nuclei and areas that are associated with regulation of energy balance include the arcuate, ventromedial, dorsomedial, paraventricular nuclei, and lateral hypothalamic area. Neurons located in these regions produce chemical messengers, which are released at their terminal fields to stimulate or inhibit feeding behavior (Meister 2007).

The hypothalamus is also a major site of food-intake regulation in birds. As in mammals, lesioning the ventromedial hypothalamus (VMH) of avian species causes hyperphagia, hyperdipsia, and obesity (Kuenzel 1994), whereas lesioning the lateral hypothalamic area decreases food intake. While these sites have traditionally been considered as satiety and feeding centers, respectively, it is currently believed that they are better considered as parts of neural circuits involved in food-intake regulation (Denbow 2000). Kuenzel (1989)

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identified five neural pathways concerned with mandibulation, taste, smell, sight, and the autonomic nervous system. Alterations in hypothalamic function which result in changes in food intake likely alter the autonomic nervous system. Increased evidence suggests that obesity results from decreased sympathetic nervous system activity. Interestingly, lines of chickens genetically selected for high body-weight did not increase food intake in response to ventromedial hypothalamic lesions, whereas low-weight birds increased food intake.

Intrahypothalamic adrenaline injections in mammals can enhance or inhibit feeding (Grossman 1962; Leibowitz 1978). In mammals, norepinephrine has been shown to both increase and decrease food intake depending on the site of injection. Injections into the ventromedial hypothalamus (VMH) or paraventricular nucleus (PVN) stimulate feeding (Leibowitz 1978), whereas injections into more lateral sites including the perifornical region decrease feeding (Leibowitz and Rossakis 1978). In poultry, norepinephrine stimulated food intake when injected into the ventromedial nucleus, paraventricular nucleus, and medial septal sites. Food intake was inhibited by injections of norepinephrine near the lateral septal organ and the anterior portion of both the nucleus reticularis superior, pars dorsalis, and the tractus occipitomesencephalicus (Denbow 1999).

In this study, we examined the possible effect of intralateral hypothalamic area (ILHy) injection of isoproterenol (a β_1- and β_2-adrenoceptor agonist) and propranolol (a non-selective β-blocker) on food and water intake in broilers.

Materials and methods

Animals

Day-old Ross 308 broiler cockerels (Eshragh Hatchery, Varamin, Iran) were housed in heated batteries. Chickens were provided with a crumble diet (Crumble #121, Chineh Feed Mill Co, Karaj, Iran) containing 21% protein and 3,200 kcal/kg metabolizable energy and water ad libitum. Experiment room temperature and relative humidity was maintained at 22 ± 2°C and 55 ± 5%, respectively. After 17 days of age onwards, the birds were taken to individual metabolic cages to get accustomed to them before the surgery. The birds were kept under continuous light during the whole study. This experiment was carried out at Physiology of Food and Fluid Laboratory, Aminabad Research Institute, Faculty of Veterinary Medicine, University of Tehran.

Chemicals

Isoproterenol (a β_1- and β_2-adrenoceptor agonist) and propranolol (a non-selective β-blocker) were purchased from Sigma-Aldrich Chemie GmbH, Germany. All solutions were prepared in pyrogen-free 0.9% NaCl solution (saline) that served as control.

Surgical preparation

When the birds weighed approximately 750 ± 30 g, they were anesthetized with 25 mg/kg iv injection of sodium pentobarbitone (Sigma-Aldrich, Germany). A 20-mm-long, 23-gauge, thin-walled stainless-steel guide cannula was implanted stereotaxically into the right lateral hypothalamic area. The stereotaxic coordinates were AP = 6.4, L = 1.0, and H = 8.5 mm below the dura mater with the head oriented as described by Van Tienhoven and Juhasz (1962). Three anchoring screws were fixed in the calvaria surrounding the cannula, and acrylic cement (Pars Acryl, Iran) was used to secure the cannula. An orthodontic # 014 wire (American Orthodontics, Sheboygan, WI, USA), trimmed to the exact length of the guide cannula, was inserted into the guide cannula while the chicks were not being used for experiments; in other words, the orthodontic wire was always in place except at the time of the injections. Penicillin (Hayan pharmaceuticals, Iran) was applied to the incision site to prevent the infection. The birds, taken to their individual full computerized metabolic cages (TSE Systems, Bad Homburg, Germany) where food and water intake can be automatically measured every minute, were allowed at least 5 days recovery following the surgery.

Experimental procedures

To determine the possible effects of ILHy microinjection of isoproterenol and propranolol on feed and water intake, six experiments were conducted. Forty-eight birds were used in each experiment. All solutions were injected on the same day during 08:00–12:00 in replicates of 12 birds, and the feeding behavior was monitored. Injections were made with a 29-gauge, thin-walled stainless-steel injection cannula which extended 0.1 mm beyond the guide cannula. The injection cannula was connected to a 2-μl Hamilton syringe via a 50-cm length of polyethylene tubing. All solutions were prepared in saline that served as the control. Beginning several days before starting the experiments, the birds were restrained daily to acclimate to the procedure. Solutions were injected over a 10-s period, and the injection cannula remained in place for an additional 30 s before removal. All birds were returned to their cages after injection. Tubing and syringes were kept in 70% ethanol, and the glassware was autoclaved to render materials pyrogen-free. Proper location of the guide cannula was verified by ILHy injection of methylene blue followed by slicing the frozen brain tissue at the end of the experiments.

Birds were deprived of food and water in all groups for 3 h prior to injections. Just after the injections fresh food (in

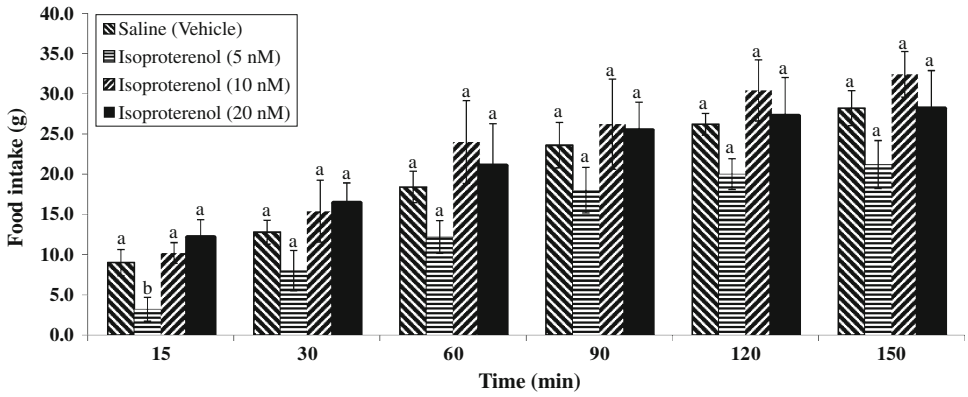

Fig. 1 Cumulative food intake (g) in 3-h food-deprived broiler cockerels following ILHy injection of saline and three doses of isoproterenol. Data are presented as mean ± SEM. *Different letters* (*a* and *b*) indicate significant differences between treatments ($P \leq 0.05$)

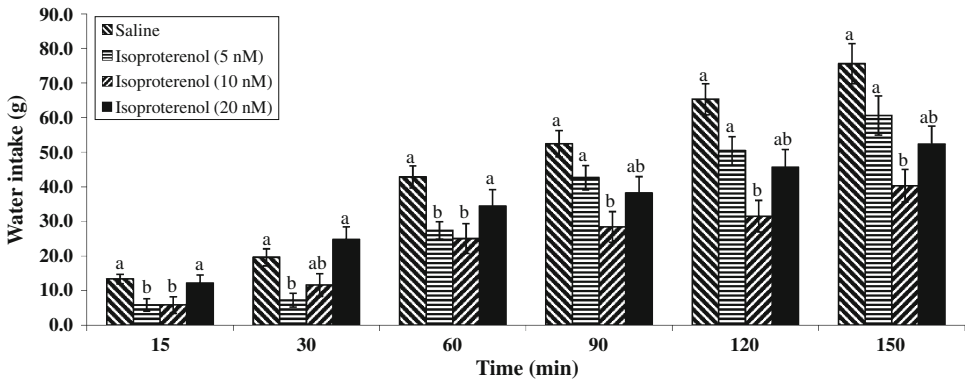

Fig. 2 Cumulative water intake (g) in 3-h water-deprived broiler cockerels following ILHy injection of saline and three different doses of isoproterenol. Data are presented as mean ± SEM. *Different letters* (*a* and *b*) indicate significant differences between treatments ($P \leq 0.05$)

Experiments 1, 3, and 5) and fresh water (in Experiments 2, 4, and 6) were provided for the birds.

In Experiments 1 and 2, isoproterenol was injected at a concentration of 0, 5, 10, and 20 nM in a volume of 2 µl into the lateral hypothalamic area. In Experiments 3 and 4, propranolol was injected at a concentration of 0, 5, 10, and 20 nM in a volume of 2 µl into the lateral hypothalamic area. Cumulative food intake (g) and water intake (g) were recorded, respectively, at 15, 30, 60, 90, 120, 150 min post-injection (PI).

In Experiment 5, cumulative food intake (g) of broiler cockerels following ILHy injection of saline, agonist, antagonist, and an equimolar combination of effective doses of agonist and antagonist (each in a 1 µl volume) was recorded at 15, 30, 60, 90, 120, and 150 min PI.

In Experiment 6, cumulative water intake (g) of broiler cockerels following ILHy injection of saline, agonist, antagonist, and an equimolar combination of effective doses of agonist and antagonist (each in a 1-µl volume) was recorded at 15, 30, 60, 90, 120, and 150 min PI.

In Experiments 1 and 2, Experiments 3 and 4, and Experiments 5 and 6, the same birds were used, and the injections were made in a 1-day interval.

Statistical analysis

Cumulative food intake (g) was subjected to one-way analysis of variance (ANOVA) at each time period. For treatments showing a main effect by ANOVA, means have been compared by post-hoc Bonferroni and Duncan's multiple range test. $P \leq 0.05$ was considered as significant difference between treatments. All data are presented as Mean ± standard error of mean (SEM).

Results

The food and water intake response to ILHy injection of isoproterenol and propranolol in broiler cockerels is presented in Figs. 1, 2, 3, 4, 5, and 6.

As it is observed in Fig. 1, ILHy injection of isoproterenol at a concentration of 5 nM significantly decreased food intake only for the first 15 min. There is a mixed response to intralateral hypothalamic area injection of different doses of isoproterenol. Isoproterenol significantly caused a short-term decrease in water intake in all concentrations used. At the concentration of 10 nM, it significantly decreased water intake even for a longer period of time (Fig. 2).

Propranolol affected food intake in all concentrations used, but at the concentrations of 5 and 10 nM its ILHy injection significantly increased food intake (Fig. 3). Propranolol at all concentrations significantly increased water intake (Fig. 4). Pretreatment with propranolol, a β-adrenergic antagonist, abolished the effect of isoproterenol on food intake (Fig. 5) and water intake (Fig. 6).

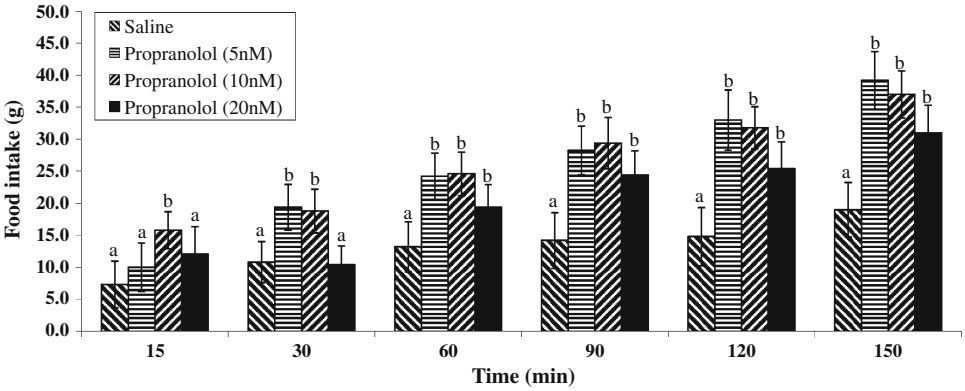

Fig. 3 Cumulative food intake (g) in 3-h food-deprived broiler cockerels following ILHy injection of saline and three different doses of propranolol. Data are presented as mean ± SEM. *Different letters* (*a* and *b*) indicate significant differences between treatments ($P \leq 0.05$)

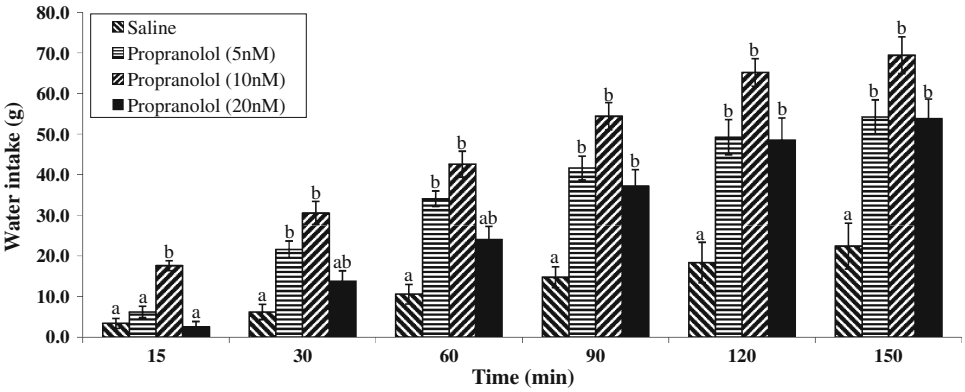

Fig. 4 Cumulative water intake (g) in 3-h water-deprived broiler cockerels following ILHy injection of saline and three different doses of propranolol. Data are presented as mean ± SEM. *Different letters* (*a*, *b*, *c*, and *d*) indicate significant differences between treatments ($P \leq 0.05$)

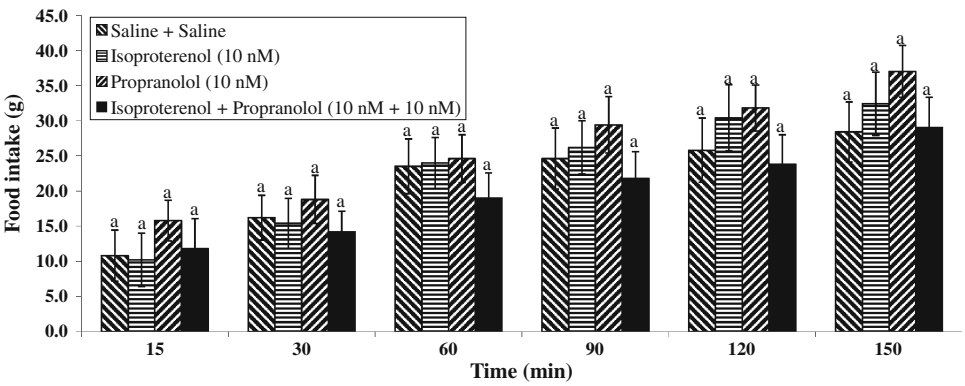

Fig. 5 Cumulative food intake (g) in 3-h food-deprived broiler cockerels following ILHy injection of saline, isoproterenol, propranolol, and an equimolar combination of effective doses of isoprterenol and propranolol. Data are presented as mean ± SEM. There is no significant difference between treatments ($P \leq 0.05$)

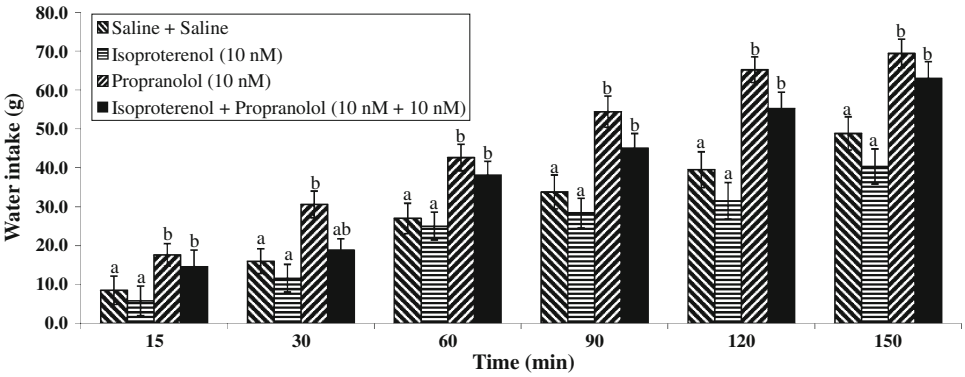

Fig. 6 Cumulative water intake (g) in 3-h water-deprived broiler cockerels following ILHy injection of saline, isoproterenol, propranolol, and an equimolar combination of effective doses of isoprterenol and propranolol. Data are presented as mean ± SEM. *Different letters* (*a* and *b*) indicate significant differences between treatments ($P \leq 0.05$)

Discussion

There is evidence that adrenergic synapses play an important role in the control of feeding (Tepperman et al. 1981; Wellman et al. 1993; Bungo et al. 1999) and drinking behavior (Glick and Greenstein 1973). Any alteration of brain noradrenaline can either increase or decrease eating, depending on the site of application and other testing variables (Wellman 2000). In mammals, ascending noradrenergic fibers from brainstem nuclei diffusely innervate the hypothalamus. Chemical and electrolytic lesions of the ascending noradrenergic fibers and route to the hypothalamus result in overeating and obesity, and application of noradrenalin into rat perifornical hypothalamus reduces eating (Wellman 2000). It has been demonstrated for years that adrenergic stimulation of different nuclei in hypothalamus modulates feeding and drinking.

The present study was designed to investigate the possible involvement of β-adrenergic circuitry on food and water intake in broiler cockerels.

The findings from experiment 1 show a significant ($P \leq 0.05$) short decrease in food intake at the lowest dose of isoproterenol applied and an increase in food intake at higher doses, none of which are statistically significant. This is parallel to the findings of Singer and Armstrong (1973) who indicated that β-agonists have an inhibitory effect on feeding. Leibowitz and Rossakis (1978) indicated that hypothalamic epinephrine-sensitive sites which shortly inhibit feeding have characteristics expected of classical β-adrenergic receptors, specifically β_2 subtype. Tachibana et al. (2003) demonstrated that the brain β_3-adrenergic receptor is involved in the inhibition of feeding in chicks.

The results from Experiment 2 are consistent with Singer and Armstrong (1973) who showed the inhibitory effect of isoproterenol on drinking in rats, and with Racotta and Soto-Mora (1993) who demonstrated the inhibitory effect of intraperitoneal injections of norepinephrine on water intake. There are inhibitory and excitatory effects of norepinephrine injected into different sites of the brain. It has been proposed that adrenergic receptors of the LHy have a dual (inhibitory and excitatory) effect on water intake (Ferrari et al. 1991). Zabik et al. (1993) supported the concept that drinking is initiated by a dopaminergically mediated thirst drive, which in turn is regulated by a noradrenergically mediated satiety system. Iyer et al. (1995) strongly suggest that water intake in response to isoproterenol is mediated in part by the rennin–angiotensin system.

The results obtained from Experiments 3 and 4 suggest that propranolol significantly increases food and water intake. Racotta and Soto-Mora (1993) suggested that blocking one type of receptors may enhance the responsiveness of the other type. We believe that occupation of β-receptors allows the endogenous epinephrine and norepinephrine to be exposed specifically to only α-receptors. It has been indicated that stimulation of α-receptors has stimulatory effect on food and water intake in mammals (Slangen and Miller 1969; Leibowitz 1975) and in avian species (Choi et al. 1995; Tachibana et al. 2009).

The observations from Experiment 5 confirm the results obtained from Experiments 1 and 2. In other words, it is concluded that β-adrenergic circuitry may not play a significant role in food intake in broilers.

The findings from Experiment 6 clearly suggest that pretreatment with propranolol abolishes the inhibitory effect of isoproterenol on water intake.

In summary, our findings suggest that ILHy β-adrenergic circuitry play an inhibitory role in food, especially in water intake. The increase in food and water intake, when propranolol alone is injected into LHy area, suggests that a β-adrenoceptor blocker not only can abolish the effect of isoproterenol on water intake but also might make more constitutive-released endogenous epinephrine and norepinephrine available for α-adrenoceptors which needs further investigations. Our findings suggest that, in broiler cockerels, ILHy β-receptors play a significant role in water intake and in short-term food intake.

It has been shown that α- and β-adrenoceptors of the LHy are possibly involved in the central mechanisms dependent on angiotensin II and subfornical organ that controls water and sodium intake (Camargo et al. 2000; Tanaka et al. 2001, 2002).

Further investigations should be carried out to examine the possible effect of β-agonists when α-adrenoceptors are blocked. As well, the role of β_3-receptors, the effect of longer period of food deprivation and the interaction between rennin–angiotensin system and adrenergic circuitry, especially in elucidating central mechanisms regulating water intake, will also be the potential subjects for future studies.

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