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The role of 5-HT_{2A} and 5-HT_{2C} receptors on harmaline-induced eating behavior in 24-h food-deprived broiler cockerels

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Summary

This study was designed to examine the effects of intracerebroventricular (ICV) injection of ketanserin (5-HT_{2a} receptor antagonist) and SB242084 (5-HT_{2c} receptor antagonist) on harmaline induced feeding and drinking response in 24-h food-deprived (FD24) broiler cockerels. At first, guide cannula was surgically implanted in the right lateral ventricle of chickens. In experiment 1, birds were injected intracerebroventriculary with 0, 25, 50 and 100 µg of harmaline. In experiment 2, chickens received 10 µg ketanserin prior to the injection of harmaline. In experiment 3, birds were administered with harmaline after 3 µg SB242084 and the cumulative food and water intake was determined at 3 h post injection. The results of this study showed that harmaline decreases food consumption and increases water intake in FD24 broiler cockerels ($P \leq 0.05$). The effect of harmaline on food and water intake was significantly attenuated with ketanserin and SB242084 pretreatment ($P \leq 0.05$). These results suggest that there is an interaction between harmaline and 5-HT (via 5-HT_{2a} and 5-HT_{2c} receptors) on eating response in chicken.

Key words: Harmaline, 5-HT, Eating response, Chicken

Introduction

β-carbolines are a class of alkaloids that have elicited considerable research interest (Pfau and Skog, 2004). They are naturally occurring alkaloids that exhibit a wide range of psychopharmacological effects because of their binding to serotonin (5-HT), benzodiazepine, imidazoline and opiate receptors as well as monoamine oxidase (MAO) inhibition (Herraiz and Chaparro, 2005; Herraiz and Chaparro, 2006a, b; Herraiz *et al*., 2008). In nature, β-carboline alkaloids are reported to be found in a number of plants, including *Banisteriopsis caapi* (*Malpighiaceae*) and *Peganum harmala* L. (*Zygophyllaceae*), their extracts exhibit psychoactive actions mediated and/or potentiated by these compounds (Callaway *et al*., 2005). The possible use of *P. harmala* in modern phyto-indole entheogen preparations is correlated to its content of β-carbolines: harmine, harmaline and tetrahydroharmine (THH), collectively known as harmala alkaloids. The interactions of harmala alkaloids at the 5-HT, dopamine and benzodiazepine receptors are a rather controversial question (Glennon *et al*., 2000). Harmaline also induces a shift in emotional reactivity, particularly in anxiety displaying anxiogenic and anxiolytic actions (Hilber and Chapillon, 2005). It is thus important to further evaluate the potential beneficial or toxic effects of these compounds, using behavioral endpoints of neural function.

On the basis of psychopharmacological effects of harmaline and its binding to 5-HT receptors as well as MAO inhibition, we hypothesized that 5-HTergic system possibly mediates harmaline signaling in the hypothalamus of birds. Thus, the present study was designed for the first time to investigate whether blocking 5-HT_{2a} and 5-

HT_{2c} receptors can influence harmaline-induced feeding and drinking response in FD24.

Materials and Methods

Animals

Day-old Ross 308 broiler cockerels (Eshragh Hatchery, Varamin, Iran) were housed in heated batteries with continuous lighting until 3 weeks of age. Birds were provided with a mash diet (21% protein and 2,869 kcal/kg of metabolizable energy) and water *ad libitum*. At approximately 2 weeks of age, the birds were transferred to individual cages. The temperature and relative humidity of the animal room were maintained at 22 ± 1°C and 50%, respectively, in addition to the continuous lighting condition. This study was approved by the ethics committee of Faculty of Veterinary Medicine of Tehran University. Animal handling and experimental procedures were performed according to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication NO.85-23, revised 1996).

Drugs

Harmaline (a MAO inhibitor), ketanserin (5-HT_{2a} receptor antagonist) and SB242084 (5-HT_{2c} receptor antagonist) were purchased from Tocris Cookson Co. (Bristol, UK). All drugs were dissolved in 1% dimethyl sulfoxide (DMSO). Control group received vehicle as control.

Surgical preparation

At 3 weeks of age, broilers were anesthetized with sodium pentobarbital (Sagatal, Rhone Merieux) (25 mg/kg body weight, iv) and a 23-gauge thin-walled stainless steel guide cannula was stereotaxically implanted into the right lateral ventricle and the stereotaxic coordinates were AP = 6.7, L = 0.7, H = 3.5-4 mm below the duramater with the head oriented. The cannula was secured with three stainless steel screws placed in the calvaria surrounding each guide cannula, then acrylic dental cement (Pars acryl) was applied to the screws and guide cannula. An orthodontic #014 wire (American Orthodontics) trimmed to the exact length of the guide cannula was inserted into the guide cannula while the chicks were not being used for experiments. Lincospectin (Razak, Tehran, Iran) was applied to the incision to prevent infection. The birds were allowed a minimum of 5 days recovery prior to injection.

Experimental procedures

To determine the possible effects of harmaline on food and water intake and its interaction with 5-HTergic system, four experiments were conducted. Twenty-four birds were used in each experiment (total used chickens for all experiments was 96). Injections were made with a 29-gauge, thin-walled stainless steel injecting cannula which extended 1.0 mm beyond the guide cannula. This injecting cannula was connected through a 60-cm-long PE-20 tubing to 10-µl Hamilton syringe. Solutions were injected over a period of 60 s. A further 60 s period was allowed to permit the solution to diffuse from the tip of the cannula into the ventricle. All experimental procedures were performed during a period from 10:00 a.m. to 02:00 p.m. Before injection, the birds were removed from their individual cages, restrained by hand, and then put back into their cages after injection. Birds were handled and injected daily during the 5-day recovery period, in order to become used to the injection procedure. Tubing and syringes were kept in 70% ethanol, and the glassware was autoclaved to render materials pyrogen-free. Three hours before the start of the experiments, animals were deprived of food and water. Immediately after injection, the birds were returned to their cages. Fresh food and water were supplied at the time of injection and cumulative food (g) and water (g) intake were recorded at 15, 30, 45, 60, 90, 120, 150 and 180 min after injection. Placement of the guide cannula into the ventricle was verified by the presence of cerebrospinal fluid and intracerebroventricular injection of methylene blue followed by slicing the frozen brain tissue at the end of the experiments.

In this study, experiment 1 was performed to examine the effect of ICV injections of harmaline on food and water

intake in FD24 (n = 6 for each group) chickens. The birds received 25, 50 and 100 µg harmaline in 10-µl vehicle. Control group was injected with 10 µl of vehicle.

In experiment 2 and 3 each bird received two injections with 15 min interval and fresh food and water were supplied at the time of second injection and cumulative food (g) and water (ml) intake were recorded at 15, 30, 45, 60, 90, 120, 150 and 180 min after the second injection.

On the other hand, in experiment 2, the first injection consisted of either 0 or 10 µg ketanserin in 5-µl vehicle. The second injection consisted of either 0 or 100 µg harmaline in 5-µl vehicle.

Experiment 3 was conducted parallel to the experiment 2, except that the chicks received 0 or 3 µg SB242084 instead of ketanserin.

The dosages of drugs were selected based on previous studies (Von Meyenburg *et al*., 2003; Medeiros *et al*., 2005; Bungo *et al*., 2008) and preliminary experiments. During the injection of higher doses of harmaline, the birds were very active, excited and sometimes convulsive.

Statistical analysis

Cumulative food and water intake were analysed by one-way analysis of variance (ANOVA), (SPSS version 15) and is presented as mean ± SEM. For treatment showing a main effect by ANOVA, means have been compared by post hoc Bonferroni test. P≤0.05 was considered as significant difference between treatments.

Results

The feeding and drinking response to intracerebroventricular injection of harmaline, ketanserin and SB242084 in broiler chickens is presented in Figs. 1-6.

In experiment 1, the injected harmaline into the lateral ventricle of FD24 chickens caused a decrease in food consumption and increase in water intake when compared with control group (Figs. 1 and 2) (P≤0.05). The anorexic and dipsogenic effects of harmaline were significant for 50 and 100 µg doses and lasted for at least 180 min. The 100 µg harmaline was selected for the following experiments because it was found to induce potent decrease in food intake and increase in water intake in FD24 animals without affecting other non-ingestive behavioral parameters.

In experiment 2, intracerebroventricular injection of 100 µg harmaline alone decreased food intake and increased water intake (P≤0.05). In addition, the effect of harmaline on cumulative food and water intake was significantly decreased by 10 µg ketanserin pretreatment (P≤0.05) (Figs. 3 and 4).

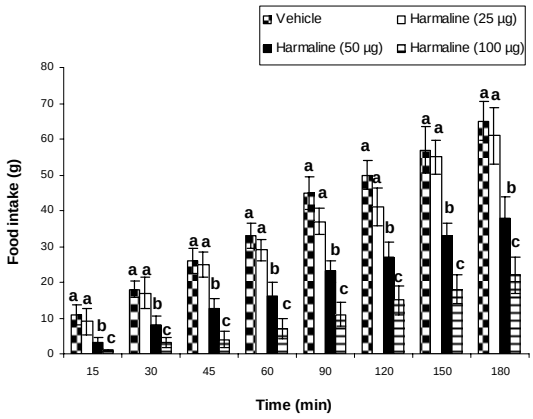

Fig. 1: Effect of intracerebroventricular injection of different doses of harmaline on food intake in 24-h food-deprived (FD24) chickens. Vehicle: 1% DMSO: Control. Data are presented as mean ± SEM. Different letters (a, b and c) indicate significant differences between treatments (P≤0.05)

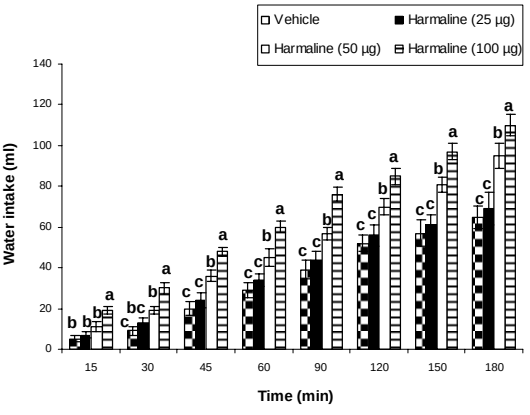

Fig. 2: Effect of intracerebroventricular injection of different doses of harmaline on water intake in 24-h food-deprived (FD24) chickens. Vehicle: 1% DMSO: Control. Data are presented as mean ± SEM. Different letters (a, b and c) indicate significant differences between treatments (P≤0.05)

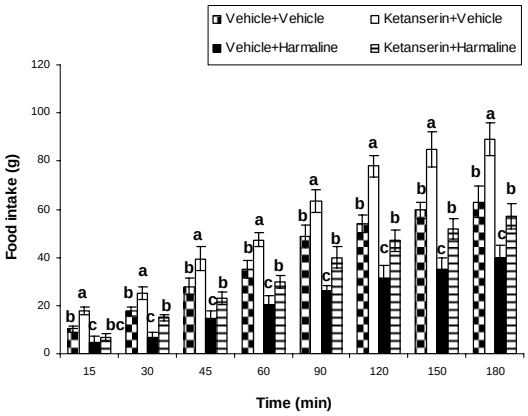

Fig. 3: Effect of intracerebroventricular injection of ketanserin (10 µg) followed by harmaline (100 µg) on food intake in 24-h food-deprived (FD24) chickens. Vehicle: 1% DMSO: Control. Data are presented as mean ± SEM. Different letters (a, b and c) indicate significant differences between treatments (P≤0.05)

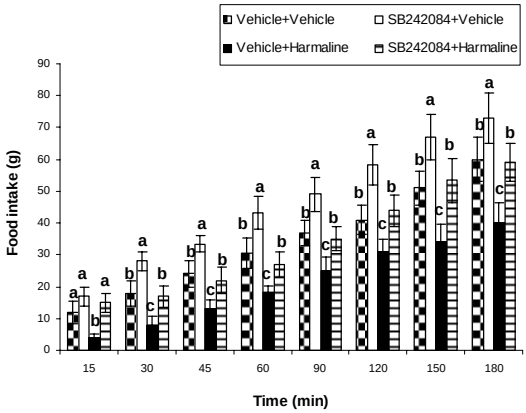

Fig. 5: Effect of intracerebroventricular injection of SB242084 (3 µg) followed by harmaline (100 µg) on food intake in 24-h food-deprived (FD24) chickens. Vehicle: 1% DMSO: Control. Data are presented as mean ± SEM. Different letters (a, b and c) indicate significant differences between treatments (P≤0.05)

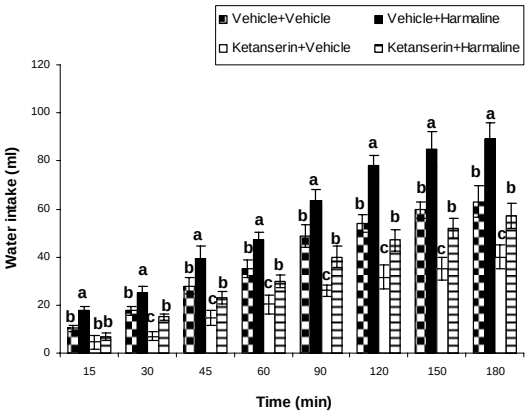

Fig. 4: Effect of intracerebroventricular injection of ketanserin (10 µg) followed by harmaline (100 µg) on water intake in 24-h food-deprived (FD24) chickens. Vehicle: 1% DMSO: Control. Data are presented as mean ± SEM. Different letters (a, b and c) indicate significant differences between treatments (P≤0.05)

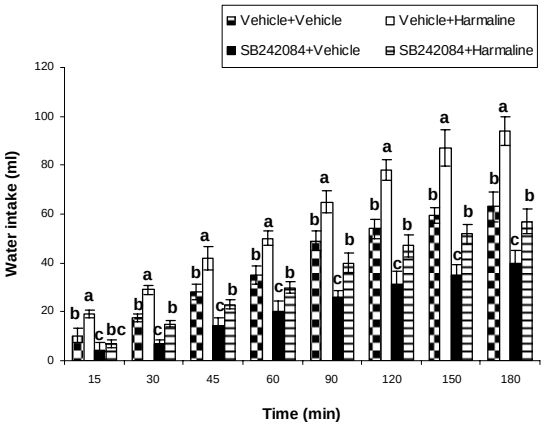

Fig. 6: Effect of intracerebroventricular injection of SB242084 (3 µg) followed by harmaline (100 µg) on water intake in 24-h food-deprived (FD24) chickens. Vehicle: 1% DMSO: Control. Data are presented as mean ± SEM. Different letters (a, b and c) indicate significant differences between treatments (P≤0.05)

In experiment 3, feeding and drinking response induced by harmaline was significantly inhibited by 3 µg SB242084 pretreatment (Figs. 5 and 6) (P≤0.05).

Discussion

The present study was designed for the first time to investigate the possible involvement of 5-HTergic circuits in harmaline control mechanisms of feeding and drinking response in broiler cockerels. The results obtained from experiment 1 showed that ICV injection of harmaline decreases food intake and increases water intake in 24 h food-deprived cockerels, and these effects of harmaline were similar to 5-HT effects on eating behavior. In this regard, inhibition of 5-HT_{2a} and 5-HT_{2c} receptors by ketanserin and SB242084 (in experiments 2 and 3, respectively) increased food intake and decreased water intake in chicks. Our

data is in line with earlier findings (Steffens *et al*., 1997) on pigeons. According to that report, ICV injection of 5-HT decreases food intake and increases water intake in 24 h food-deprived pigeons. Furthermore, ICV injection of 5-HT in Leghorn species decreases food intake in both satiate and food-deprived birds, but water intake just in satiate birds was increased and remained equable in food-deprived birds (Denbow *et al*., 1983). Such discrepancies in water intake may be due to the lineage properties, the affected site in the brain or 24 h deprivation of food in this experiment. These results indicate that lineage properties are important in the aminergic feeding and drinking regulatory systems. In another study, ICV injection of 5-HT in heavy weight and light weight chicken's lines showed no changes in food intake in satiate birds but decreased the food intake in 24 h food-deprived birds in both lines with prolonged influence on heavy weight line (Denbow *et al*., 1986) and this effect of 5-HT on feeding behavior is mediated by 5-HT_{2c} receptor (Cedraz-Mercez *et al*., 2005). In experiments 2 and 3, the decreased food consumption and increased water intake induced by the ICV injection of harmaline was attenuated by pretreatment with ketanserin and SB242084, respectively (Figs. 3-6). Our findings showed that the effect of harmaline on food and water intake is probably modulated by the pathway(s) linked to the 5-HT_{2a} and 5-HT_{2c} receptors. On the other hand, harmaline and serotonin may converge on the same signal transduction mechanisms for induction of eating response in regulatory centers of appetite. It is well known that central administration of 5-HT induces anorexia in chickens (Denbow *et al*., 1986; Sashihara *et al*., 2002) and rats (Hewson *et al*., 1988) by 5-HT_{2c} receptors. We also found that the harmaline-induced anorexia and polydipsia is attenuated by the 5-HT_{2a} and 5-HT_{2c} receptors antagonists (Figs. 3-6). Thus, it seems that harmaline injected into the brain of chicks stimulate 5-HTergic neurons and induced hypophagia and polydipsia via 5-HT_{2a} and 5-HT_{2c} receptors.

Several lines of evidence suggest that 5-HTergic system may be involved in the harmaline-induced regulation of feeding and drinking behavior. Harmaline is a competitive and reversible inhibitor of monoamine oxidase type-A (MAO-A) enzymes, and are believed to inhibit 5-HT reuptake (Kim *et al*., 1997). On the other hand, harmala alkaloids reduce or prevent the first pass metabolism of the hallucinogenic amine(s) such as 5-HT by inhibiting MAO-A activity, thus 5-HT is accumulated in synaptic cleft (McKenna *et al*., 1984). Additionally, the harmala alkaloids themselves are also psychoactive if their dosage is sufficient, possibly due to direct activation of 5-HT_{2c} receptors (Efron *et al*., 1967). As noted, 5-HTergic system possibly mediates harmaline signaling in the hypothalamus by two mechanisms: one, harmaline is a MAO inhibitor and also inhibits 5-HT reuptake, so it can increase the levels of 5-HT in synaptic spaces and the other, harmaline directly activates 5-HT_{2c} and 5-HT_{2c} receptors in regulatory centers of feeding and drinking balance. Thus, harmaline probably influences food and water intake via 5-HT_{2a} and 5-HT_{2c} receptors in regulatory centers of eating behavior.

In summary, the results of the current study suggest that the harmaline and 5-HT systems can function together by mobilizing intracellular signal transduction pathways in the target sites to balance feeding and drinking. These drugs may activate common orexigenic and anorexigenic networks within or outside the hypothalamus to modulate eating behavior. Elucidation of the underlying cellular and molecular mechanisms in the interplay between harmaline and 5-HT in hypothalamic neurons needs further investigation.

Acknowledgement

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