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Diencephalic and septal structures containing the avian vasotocin receptor (V1aR) involved in the regulation of food intake in chickens, *Gallus gallus*

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HIGHLIGHTS

- The avian V1aR in brain regulates food intake.
- V1aR antagonist reduces AVT and CRH neuronal activity.
- Magnocellular, arginine vasotocin neurons are associated with food intake.
- V1aR containing glial cells may modulate CRH neurons.

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ABSTRACT

Recently, it was found that the avian central vasotocin receptor (V1aR) is associated with the regulation of food intake. To identify V1aR-containing brain structures regulating food intake, a selective V1aR antagonist SR-49059 that induced food intake was administrated intracerebroventricularly in male chickens followed by detection of brain structures using FOS immunoreactivity. Particularly, the hypothalamic core region of the paraventricular nucleus, lateral hypothalamic area, dorsomedial hypothalamic nucleus, a subnucleus of the central extended amygdalar complex [dorsolateral bed nucleus of the stria terminalis], medial septal nucleus and caudal brainstem [nucleus of the solitary tract] showed significantly increased FOS-ir cells. On the other hand, the supraoptic nucleus of the preoptic area and the nucleus of the hippocampal commissure of the septum showed suppressed FOS immunoreactivity in the V1aR antagonist treatment group. Further investigation revealed that neuronal activity of arginine vasotocin (AVT-ir) magnocellular neurons in the supraoptic nucleus, preoptic periventricular nucleus, paraventricular nucleus and ventral periventricular hypothalamic nucleus and most likely corticotropin releasing hormone (CRH-ir) neurons in the nucleus of the hippocampal commissure were reduced following the antagonist treatment. Dual immunofluorescence labeling results showed that perikarya of AVT-ir magnocellular neurons in the preoptic area and hypothalamus were colabeled with V1aR. Within the nucleus of the hippocampal commissure, CRH-ir neurons were shown in close contact with V1aR-ir glial cells. Results of the present study suggest that the V1aR plays a role in the regulation of food intake by modulating neurons that synthesize and release anorectic neuropeptides in the avian brain.

1. Introduction

Behavioral responses in both mammals and birds are determined by internal and external cues [1,2] and sensory integration is necessary in order to effect appropriate behavioral responses that ensure the survival of individuals. Foraging and food intake are behavioral responses initiated by deficiency of energy and nutrients [3–5]. The basic food intake response is perturbed during stress that has a significant effect on growth. Food intake and stress responses are regulated by neurohormones and/or neuromodulators that are synthesized and released by neurons in the hypothalamus of vertebrates [6,7]. Several neurohormones are involved in the regulation of stress [6,8] and some of them, such as corticotropin releasing hormone (CRH), arginine vasopressin (AVP) and pro-opiomelanocortin, are known for their anorectic effect [7,9,10].

In birds, arginine vasotocin (AVT), homologous to mammalian AVP, is a neurohormone that regulates several physiological and behavioral responses [11–15] and its functions have been extensively reviewed

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[16,17]. Vasotocin containing neurons are almost exclusively present in the preoptic area and hypothalamus including the supraoptic nucleus (SO), preoptic periventricular nucleus (POP), paraventricular nucleus (PVN), periventricular hypothalamic nucleus (PHN) and with few exceptions including dorsolateral thalamus, nucleus of the stria terminalis, and perirotundic area [18,19]. Both parvocellular and magnocellular AVT-ir neurons are involved in neuroendocrine regulation and upon their activation AVT is released either into the median eminence (from parvocellular AVT neurons) or the neurohypophysis (from magnocellular neurons). Similar to AVP in mammals, AVT regulates the hypothalamic-pituitary-adrenal (HPA) axis [20–22] and is involved in physical, social and psychological stress [23–26]. Vasotocin released in response to stress has a direct effect on the HPA and augments the effect of stress along with CRH [20,21,27]. Being a stress hormone, AVT also appears to affect food intake. Interestingly, both endogenous and exogenous analogs of AVP and AVT have been reported to suppress food intake in both mammals [28,29] and birds [30,74], respectively. Thus, AVT acts as a stress hormone as well as an anorectic agent. Furthermore, rodent studies have shown that food deprivation for an extended period of time significantly reduced AVP expression in the hypothalamus [31,32]. Hence, AVT may play a short term role in the central regulation of food intake and during a long period of food deprivation AVT is expected to be suppressed.

Although, AVT is released via its axonal terminals in the median eminence or the neurohypophysis, the nonapeptide is also found to be released within the brain [33] to modulate central responses. This concept concurs with the effects induced from central administration of analogs of AVT [15,30,74]. Specific functions of AVT in birds are mediated through its four known receptor types. Depending on localization, each receptor type has distinct functions ranging from osmoregulation to reproduction and social bonding to stress [34–36]. Behavioral and physiological roles of the nonapeptide have been extensively studied through the application of agonists or antagonists to specific receptors or using knockout animal models and related techniques (for review see [37]).

Several lines of evidence suggest that in mammals food intake is mediated through V1a receptors [29,38,39]. In birds, a specific function of vasotocin receptors in the regulation of food intake remains controversial. Two lines of evidence support the avian central V1aR involvement in food intake. Specifically, the avian V1aR and not V1bR (previously termed the VT4R and VT2R, respectively; for the change in receptor nomenclature see reference [42]) was reported in the chicken brain [40,41] including strong V1aR immunoreactivity in glial cells surrounding circumventricular organs and moderate to weak V1aR immunoreactivity in diencephalic structures such as the SO and ventral PHN [41]. Hence, neurons within these structures containing V1aR immunoreactivity are likely involved in the regulation of food intake. Secondly, intracerebroventricular (ICV) administration of a selective V1aR antagonist (SR-49059) [51,52] not only attenuated stress levels but also increased food intake in chicks [42]. It was based on this assumption that anorectic neurohormones, such as CRH and AVT, associated in the initiation of stress response could be blocked by the antagonist resulting in an increase in food intake. Moreover, ICV administration of a V1aR antagonist also augmented food intake induced by neuropeptide Y (NPY) further supporting the premise that anorectic effects of stress neurohormones could be blocked by the V1aR antagonist to facilitate the additional increase in food intake [42].

Therefore, a study was conducted to identify V1aR containing brain structures associated with the regulation of food intake. Thus the central role of V1aR on food intake was examined using ICV administration of SR-49059 followed by FOS immunoreactivity in brain structures. As a positive control, neuropeptide Y (NPY), a known potent orexigenic peptide in vertebrates [43,44], was used in order to compare brain structures associated with the regulation of food intake in birds. Furthermore, in the present study because of the changes observed in FOS-ir cell counts following ICV administration of a V1aR antagonist, dual immunohistochemistry was also performed to identify the signature of neuronal phenotypes.

2. Materials and methods

Day-old male Cobb 500 chicks were obtained from a commercial hatchery and raised in brooder cages set at 32 °C with a weekly 2.5 °C reduction in temperature until 21 °C was reached and maintained until the end of the experiment. Birds had access to a standard chick starter feed (22% protein, metabolizable energy was 3100 kcal/kg) and water ad libitum. After two weeks of age, birds with similar body weight were randomly selected for the study. All of the procedures in experiments were approved by the University of Arkansas Institutional Animal Care and Use Committee.

2.1. Intracerebroventricular injections

At 4 weeks of age, a stainless steel cannula was implanted into the lateral ventricle of each bird as previously described [26,27]. Birds were allowed to recover for at least 4 days and the cannula position in each bird was tested with an ICV dose (80 ng) of angiotensin II (Human ANG II, Sigma Aldrich, St. Louis, MO), a potent dipsogen [45]. Birds that displayed binge drinking behavior within 3 min were randomly selected and placed in individual cages for the study. Later, birds were injected with one of the three treatments: 1) control (physiological saline 0.9%), 2) V1aR antagonist SR-49059 (250 ng/bird) or 3) NPY (4 μg/bird). Doses of SR-49059 (Sigma-Aldrich, St. Louis, MO) and NPY (Bachem, Torrance, CA) were determined in a previous study [42]. Sterile physiological saline was used as the diluent for preparing SR-49059 or NPY and were administered ICV in 8 μl volume over a minute period ($n = 5$/treatment). Following ICV administration, birds were returned to their home cages with feeder removed and their behavior was video recorded for 1 h using a Sony HD PJ430V camcorder. Thereafter, birds had access to food for 1 h. Birds were then anesthetized with sodium pentobarbital solution (27 mg/kg, i.v.), perfused via carotid arteries using ice cold 0.1 M phosphate buffer (PB) and ice cold Zamboni fixative. The calvarium of each skull was removed and brains were blocked using a stereotaxic instrument (Kopf, Tujunga, CA). The blocked brains were removed from the skull and placed in the same fixative overnight. Thereafter, brains were transferred to a sucrose solution (30% in 0.1 M PB) until they sank. After saturation each sample was removed from the sucrose solution and stored at −80 °C until sectioned.

2.2. Behavioral assessments

Video records were used to manually score the following behavioral parameters during the first hour: (1) foraging or frequency of food searching behavior, (2) resting bouts, and (3) amount of time spent drinking water. Because water was provided through drinkers attached to the water lines passing through all cages, volume of water consumed by each bird could not be recorded. During the second hour of post-injection food intake was measured to the nearest 0.1 g.

2.3. Immunocytochemistry (ICC)

Brains were sectioned in the coronal plane at 40 μm thickness and sections were stored at −20 °C in ethylene glycol based cryoprotective solution [75] until use. Immunocytochemistry for FOS was performed as described [26,46]. Because of changes observed in FOS-ir cell counts in the SO dual FOS/AVT immunocytochemistry was also performed. Briefly, sections were rinsed several times in 0.02 M phosphate buffered saline (PBS) and treated with 0.2% hydrogen peroxide solution to block peroxidase activity, followed by treatment with 0.4% TritonX-100 for 20 min. Sections were incubated in 5% normal goat serum for 30 min and then with anti-FOS primary antibody raised in rabbit (sc-253, LOT#C2112, Santa Cruz Biotechnology; 1:3000) for at least 24 h at 4 °