Hormones and Behavior 60 (2011) 327–335

Contents lists available at ScienceDirect

Hormones and Behavior

journal homepage: www.elsevier.com/locate/yhbeh

Dopamine and mesotocin neurotransmission during the transition from incubation to brooding in the turkey

A. Thayananuphat [a], O.M. Youngren [a], S.W. Kang [a], T. Bakken [a], S. Kosonsiriluk [a], Y. Chaiseha [b], M.E. El Halawani [a,*]

[a] Department of Animal Science, University of Minnesota, St. Paul, MN 55108, USA
[b] School of Biology, Institute of Science, Suranaree University of Technology, Muang District, Nakhon Ratchasima, 30000, Thailand

ARTICLE INFO

Article history:
Received 13 May 2011
Revised 17 June 2011
Accepted 20 June 2011
Available online 30 June 2011

Keywords:
Dopamine
Mesotocin
Birds
Incubation behavior
Brooding behavior

ABSTRACT

We investigated the neuroendocrine changes involved in the transition from incubating eggs to brooding of the young in turkeys. Numbers of mesotocin (MT; the avian analog of mammalian oxytocin) immunoreactive (ir) neurons were higher in the nucleus paraventricularis magnocellularis (PVN) and nucleus supraopticus, pars ventralis (SOv) of late stage incubating hens compared to the layers. When incubating and laying hens were presented with poults, all incubating hens displayed brooding behavior. *c-fos* mRNA expression was found in several brain areas in brooding hens. The majority of *c-fos* mRNA expression by MT-ir neurons was observed in the PVN and SOv while the majority of *c-fos* mRNA expression in dopaminergic (DAergic) neurons was observed in the ventral part of the nucleus preopticus medialis (POM). Following intracerebroventricular injection of DA or oxytocin (OT) receptor antagonists, hens incubating eggs were introduced to poults. Over 80% of those injected with vehicle or the D1 DA receptor antagonist brooded poults, while over 80% of those receiving the D2 DA receptor antagonist or the OT receptor antagonist failed to brood the poults. The D2 DA/OT antagonist groups also displayed less *c-fos* mRNA in the dorsal part of POM and the medial part of the bed nucleus of the stria terminalis (BSTM) areas than did the D1 DA/vehicle groups. These data indicate that numerous brain areas are activated when incubating hens initially transition to poult brooding behavior. They also indicate that DAergic, through its D2 receptor, and MTergic systems may play a role in regulating brooding behaviors in birds.

Introduction

Two essential events occur in an incubating turkey hen when its eggs hatch. Within 24 h, circulating prolactin (PRL) levels decline sharply (Opel and Proudman, 1989) and the hen leaves the nest and begins to display brooding behavior toward its new offspring (Wentworth et al., 1983). Dopamine (DA) and the D2 DA receptor have been implicated in the inhibition of *in vitro* PRL secretion (Xu et al., 1996). Elevated DA dosage, infused intracerebroventricularly (ICV), or reduced doses, infused into the anterior pituitary, prevented the elevated plasma PRL observed after electrical stimulation in the medial preoptic area (MPOA) (Youngren et al., 1995, 1998). Infusion of a D2 DA receptor antagonist into the anterior pituitary also inhibited electrically-driven PRL release (Youngren et al., 1998). Fairly discrete groups of DA neurons are scattered throughout the hypothalamus, specifically in the nucleus preopticus medialis (POM), nucleus paraventricularis magnocellularis (PVN), regio lateralis hypothalami (LHy), nucleus mamillaris lateralis (ML), and nucleus premamillaris (PMM) (Appeltants et al., 2001; Bailhache and Balthazart, 1993; den Boer-Visser and Dubbeldam, 2002). However, the DA group or groups responsible for regulating or inhibiting PRL secretion are not known.

Oxytocin (OT) and DA are two of the key neurochemicals involved in maternal behavior in mammals. OT seems to be involved during the onset of maternal behavior. The ICV infusion of OT induced maternal behavior to appear in estrogen-primed virgin rats (Pedersen et al., 1982), and ICV injections of OT antagonist or antisera prevented or delayed the onset of maternal behavior (Pedersen et al., 1985; van Leengoed et al., 1987).

Mesotocin (MT) is the avian homolog of OT (Acher et al., 1970). MT-immunoreactive (ir) neurons were found in several brain areas, such as the nucleus supraopticus, pars ventralis (SOv) and PVN (Bons, 1980; Goossens et al., 1977). The function of MT in birds is not well understood, although it does not appear to be involved in aggression, partner preference, cardiovascular function, and plasma osmolarity (Goodson et al., 2004; Robinzon et al., 1994), but may participate in renal blood flow (Bottje et al., 1989). The role of MT in avian brooding behavior has never been investigated.

DA has been shown to be involved in the onset and maintenance of maternal behavior in mammals (Lonstein et al., 2003; Silva et al.,

* Corresponding author at: Department of Animal Science, University of Minnesota, 495 An Sci/Vet Med Bldg, 1988 Fitch Ave., St. Paul, MN 55108, USA. Fax: +1 612 625 2743.
E-mail address: elhal001@umn.edu (M.E. El Halawani).

0018-506X/$ – see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.yhbeh.2011.06.009

2001). Injection of D1 DA and D2 DA receptor antagonists in periparturient rats attenuated maternal behavior (Byrnes et al., 2002), and interrupted ongoing maternal behavior in lactating rats (Silva et al., 2001). Reports of the effects of DA during brooding behavior in birds are limited. DA activity increased in the hypothalamus of parenting ring doves, and an increase in the number of DA neurons was observed in the periventricular nucleus and anterior dorsomedial nucleus of the thalamus (Lea et al., 2001), suggesting a possible role for DA in avian brooding behavior.

Several studies have demonstrated that the MPOA and bed nucleus of the stria terminalis (BST) are important for maternal behavior. Lesioning in the MPOA and ventral part of the BST disrupted maternal behavior in the rat (Cohn and Gerall, 1989; Numan and Numan, 1996). Estradiol injections promoted maternal behavior and enhanced Fos protein expression in the MPOA and BST (Sheehan and Numan, 2002). In rats, the MPOA, and BST expressed Fos protein, a marker for neuronal activation, after mothers were reunited with their pups (Lin et al., 1998; Numan et al., 1998). Immediate-early gene transcriptions have also been used to confirm the importance of the POM and medial part of the BST (BSTM) in avian brooding behavior. In Japanese quail, the density of Fos-like ir neurons was elevated in the BSTM and ectostriatum in brooding females, whereas ZENK-ir cells decreased in the POM in female Japanese quail that did not show brooding behavior (Ruscio and Adkins-Regan, 2004). However, the neurotransmitter(s) involved in these areas during the transition to brooding behavior have never been identified.

To verify the location of DA and MT neurons and their involvement in the incubating–brooding transition, immunocytochemistry (ICC) was used to reveal tyrosine hydroxylase-ir (TH-ir) and MT-ir neurons in late stage of incubating hens and hens that were laying eggs regularly. Incubating turkey hens were stimulated for maternal behavior by introducing them to one week old poults. The hens were observed for maternal behavior and hypothalamic areas that were activated following poult stimulation were also mapped by using *in situ* hybridization (ISH) to detect *c-fos* mRNA expression. ICC and ISH were combined to double-labeled *c-fos* mRNA expression in TH-ir and MT-ir neurons. To further verify the importance of the DAergic and MTergic systems in brooding behavior, hens were centrally injected with either D1 DA, D2 DA, or OT receptor antagonists prior to their stimulation for maternal behavior.

Experimental procedures

Experimental animals

Adult large white female Hybrid turkeys were housed in floor pens with nest boxes under long day lighting conditions (14 h of light and 10 h of dark; 14L:10D). Food and water were available *ad libitum*. All poults used in Experiments 2 and 3 were housed together in a heated brooder. All animals were treated in accordance with University of Minnesota Institutional Animal Care and Use Committee Guidelines.

Experiment 1: MT and DA neuronal systems in laying and incubating hens

Hens were divided into two groups with six animals in each group: (1) hens that had been laying eggs for 4 weeks, and (2) incubating hens that had been observed sitting on the nest at least six times each day without laying eggs for 4 weeks (*i.e.* the duration of incubation in turkeys).

Tissue preparation

Hens were intravenously injected with heparin (American Pharmaceutical Partners, Inc., Los Angeles, CA), then euthanized with intravenous sodium pentobarbital (Euthasol, Virbac AH, Inc., Fort Worth, TX). Heads were decapitated and immediately pressure-perfused *via* both carotid arteries with phosphate buffered saline (PBS, pH 7.4) for 3–5 min, followed by a fresh fixative solution containing 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) for 35 min. Brains were soaked in 20% sucrose in PBS at 4 °C until saturated for cryoprotection. Then brains were frozen in powdered dry ice for 1 h, and stored at −80 °C until sectioned at 16 μm with a cryostat. Sections were stored desiccated at −80 °C until further processed.

The specificity of the antiserum to MT

The primary antiserum used to detect MT in this experiment (a gift from Dr. Seiishiro Kawashima, University of Tokyo, Japan) was a rabbit polyclonal antibody raised against OT. This antiserum was proved to cross react with isotocin, a homolog of OT in fish (Ota et al., 1996) and used to stain MT neurons in the quail hypothalamus (Ukena et al., 2003). However, no specificity of this antiserum to MT was verified. Therefore, the pre-absorption test was performed to verify the specificity of this antiserum to MT. Tissue sections were treated with normal rabbit serum, OT antiserum diluted 1:1000 with PBS and 1% bovine serum albumin pre-absorbed with MT (Bachem, Torrance, CA) 10 μg/ml overnight, or OT antiserum diluted 1:1000 with PBS and 1% serum albumin. Also, the specificity of this antiserum to MT, but not vasotocin (VT) was verified by investigating the distribution of immunoreactivity in adjacent tissue sections stained with this antiserum or VT antiserum (Haruta et al., 1991).

ICC

Every sixth slide of tissue sections was treated with PBS (pH 7.4) for 10–30 min, and incubated with a rabbit polyclonal antibody raised against OT or a mouse monoclonal antibody raised against TH (ImmunoStar, Hudson, WI), and validated for use in the turkey (Kang et al., 2007), diluted 1:1000 with PBS containing 0.3% triton X-100 and 1% bovine serum albumin at 4 °C overnight. After incubation, tissues were washed three times in cold PBS and incubated with Cy3 anti-rabbit IgG (Jackson ImmunoResearch, West Grove, PA), or Cy3 anti-mouse IgG (Jackson ImmunoResearch, West Grove, PA) diluted 1:500 with PBS containing 0.3% triton X-100 and 1% bovine serum albumin at room temperature for 1 h. Tissues were then washed three more times and mounted with DPX mountant (Sigma-Aldrich, St Louis, MO) before coverslips were applied.

Numbers of MT-ir and TH-ir neurons

The number of MT-ir or TH-ir neurons was counted under a Nikon microscope with a magnification of 200×. Only ir cell bodies exhibiting visible nuclei or showing the appropriate shape (round and smooth-edged) were considered and counted. Results were expressed as mean values ± SEM. A total value of MT-ir or TH-ir neurons was compared between groups by using Student's *t*-test. Differences were considered significant if the p-value was less than 0.05.

Experiment 2: Brooding behavior and c-fos mRNA expression

Hens that had been incubating eggs for 4 weeks, the period required for poults to hatch and the initiation of brooding behavior were divided into two groups with six animals in each group: (1) experimental group: hens in which brooding behavior was induced by replacing eggs with 10 one-week old poults. Brooding behaviors; calling to poults, encouraging poults to go underneath her wings, a crouching posture, and feather fluffing were observed. Brains were collected after a 30 min period of poult exposure as described in Experiment 1. (2) Control group: hens at the same stage of incubation that were not exposed to poults. Instead, eggs were handled by removing and placing them back under hens. The control group was processed prior to the treatment group to eliminate confounding effects from hearing or seeing poults. Tissue preparation was performed as described in Experiment 1.