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Differential roles of hypothalamic serotonin receptor subtypes in the regulation of prolactin secretion in the turkey hen

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ABSTRACT

In the turkey, exogenous serotonin (5-hydroxytryptamine, 5-HT) increases prolactin (PRL) secretion by acting through the dopaminergic (DAergic) system. In the present study, infusion of the 5-HT_{2C} receptor agonist, (R)(−)-DOI hydrochloride (DOI), into the third ventricle stimulates PRL secretion, whereas the 5-HT_{1A} receptor agonist, (+/−)-8-OH-DPAT hydrobromide (DPAT), inhibits PRL secretion. Using the immediate-early gene, *c-fos*, as an indicator of neuronal activity, *in situ* hybridization histochemistry showed preferential *c-fos* co-localization within tyrosine hydroxylase immunoreactive neurons (the rate limiting enzyme in DA synthesis) in the areas of the *nucleus preopticus medialis* (POM) and the *nucleus premammillaris* (PMM), in response to DPAT and DOI, respectively. To clarify the involvement of 5-HT_{1A} and 5-HT_{2C} receptors in PRL regulation, their mRNA expression was determined on hypothalamic tissue sections from birds in different reproductive stages. A significant difference in 5-HT_{1A} receptor was observed, with the POM of hypoprolactinemic short day and photorefractory birds showing the highest expression. 5-HT_{2C} receptors mRNA did not change during the reproductive cycle. The data presented support the notion that DA neurons in the PMM and POM mediate the stimulatory and inhibitory effects of 5-HT, respectively, on PRL secretion and the 5-HTergic system can both stimulate and inhibit PRL secretion.

Introduction

Serotonin (5-hydroxytryptamine, 5-HT) has been shown to be one of several neurotransmitters involved in the regulation of prolactin (PRL) secretion in turkeys (Fehrer et al., 1985; El Halawani et al., 1988). Infusion of 5-HT into the third ventricle causes the release of PRL (Hargis and Burke, 1984). 5-HT acts through dopamine (DA) and the avian PRL-releasing factor, vasoactive intestinal peptide (VIP) to affect PRL secretion (El Halawani et al., 1995; Youngren et al., 1998). DA can have both stimulatory and inhibitory effects on PRL, depending on its site of action (Chaiseha et al., 2010). Acting on the D_2 DA receptors of pituitary lactotropes, DA inhibits PRL release, even in the presence of VIP (Youngren et al., 2002). VIP is unable to stimulate PRL release from cultured pituitary cells incubated in the presence of physiological inhibitory levels of DA in mammalian and avian species (Mogg and Samson, 1990). At the hypothalamic level, DA interacts with the D_1 DA receptors to stimulate VIP release. D_1 DA receptors localized on VIP neurons have been found in the infundibular nuclear complex (INF; Chaiseha et al., 2003). Thus, 5-HT activates DA neurons which synapse with and activate VIP neurons in the INF to stimulate PRL secretion (El Halawani et al., 2001).

As with DA receptors, different subtypes of 5-HT receptors can have both stimulatory and inhibitory influences on PRL secretion. In mammals, PRL secretion is shown to be regulated through 5-HT_{1A} and 5-HT_{2C} receptors (Kellar et al., 1992; Albinsson et al., 1994). 5-HT appears to act centrally and not directly at the pituitary level to stimulate PRL secretion in birds (Fehrer et al., 1985; Macnamee and Sharp, 1989; El Halawani et al., 1995; Pitts et al., 1996). There are indications that 5-HT_{1A} and 5-$HT_{2A/2C}$ receptor subtypes mediate

Abbreviations: 5-HT, serotonin (5-hydroxytryptamine); DA, dopamine; DPAT, (+/−)-8-OH-DPAT hydrobromide; DOI, (R)(−)-DOI hydrochloride; ES, electrical stimulation; IHC, immunohistochemistry; INC, incubating; INF, infundibular nuclear complex; ISH, *in situ* hybridization; LAY, laying; ME, median eminence; ML, *nucleus medialis lateralis*; NPS, non-photostimulated; PBS, phosphate buffered saline; PMM, *nucleus premammillaris*; POA, preoptic area; POM, *nucleus preopticus medialis*; PRL, prolactin; REF, photorefractory; SSC, saline sodium citrate; TH, tyrosine hydroxylase; VIP, vasoactive intestinal peptide.

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5-HT inhibition and stimulation, respectively, on target neurons in birds (Niitsu et al., 1995; Herremans et al., 1999). More recently, both receptor subtypes have been shown to be involved in regulating PRL secretion in the turkeys. Infusion of the 5-HT_{2C} receptor agonist, (R)(−)-DOI hydrochloride (DOI), into the third ventricle causes PRL release, while infusion of the 5-HT_{1A} receptor agonist, (+/−)-8-OH-DPAT hydrobromide (DPAT), inhibits this PRL release (Chaiseha et al., 2010). DA neurons in the *nucleus preopticus medialis* (POM) have been shown to project to the median eminence (ME) in pigeons (Berk and Butler, 1981). It is possible that 5-HT inhibits PRL secretion by activating DAergic neurons in the POM projecting to the ME and expressing 5-HT_{1A} receptors, or stimulates PRL secretion by activating DAergic neurons in the *nucleus premammillaris* (PMM) projecting to the INF and expressing 5-HT_{2C} receptors.

The aims of this study were to determine the roles of DA and VIP neurons in 5-HT regulated PRL secretion and to describe the distribution of 5-HT_{1A} and 5-HT_{2C} receptor mRNA expressions in the turkey hypothalamus in several hypothalamic regions at different reproductive stages.

Materials and methods

Experimental animals

Somatically mature female Hybrid turkey hens (*Meleagris gallopavo*), weighing 8–10 kg at 28–30 weeks of age, were used. The hens were housed in light controlled rooms with food and water available *ad libitum*. All hens were housed and used in accordance with University of Minnesota Institutional Animal Care and Use Committee Guidelines.

Experiment 1: activation of DA and VIP neurons involved in 5-HT regulated PRL secretion in the turkey

Twenty four female laying Hybrid turkeys were used. Birds were anesthetized with a xylazine–ketamine HCl solution (1 mg xylazine:25 mg ketamine) given intravenously and the brachial vein in each wing was cannulated to facilitate further anesthesia and blood sample collection. The head of the anesthetized turkey was held in a stereotaxic instrument (David Kopf Instruments, Tujunga, CA, USA, Model 1204, with chicken beak adaptor). The beak adaptor was set to a point 23.0 mm below the vertical zero of the instrument, and the beak clamp adjusted to fit snugly against the frontal process (snood).

Concentrations of DOI and DPAT were selected based on previous experiments (Chaiseha et al., 2010) showing them effective in stimulating or inhibiting PRL secretion, respectively. DOI or DPAT was dissolved in oxygen-free boiled water immediately before use. DOI, DPAT, or vehicle was infused using a 28-gauge stainless steel internal cannula within a 22-gauge stainless steel guide cannula placed within the third ventricle. The infusion cannula was connected by polyethylene tubing to a microsyringe driven by an infusion pump (Harvard Apparatus, South Natick, MA, USA, Model 906). The pumping rate was 10 nmol/min. During filling of the infusion cannula and tubing, an air bubble was placed between the distilled water that filled the infusion system and the infusate that was aspirated up into it. This bubble served not only to separate the infusate from the distilled water, but also served as away to monitor the infusion rate.

The experimental protocol consisted of a 20 min control period during which no treatment was infused (blood sampled at −20, −10, −5, and 0 min) followed by a 40-min infusion period (blood sampled at 5, 10, 15, 20, 25, 30, 35, 40 min). Eight birds were infused with DOI, eight were infused with DPAT, and eight were infused with the oxygen-free boiled water-vehicle. Peripheral blood samples were collected for determining plasma PRL levels. In order to take repeated blood samples, a polyethylene tube (PE-160, Intramedic, Clay Adams, Parsippany, NJ, USA) was inserted 10 cm into the brachial vein of the wing and secured with ties. A stopcock and two syringes allowed for both withdrawal of blood and flushing of the cannula with physiological saline. Withdrawn blood (2.5 ml per sample) was replaced with an equal volume of saline and the cannula remained full of saline between drawings.

After the infusion, birds were intravenously injected with heparin (American Pharmaceutical Partners, Los Angeles, CA, USA), then euthanized with intravenous sodium pentobarbital (Euthasol, Virbac AH, Fort Worth, TX, USA). Birds were decapitated and immediately pressure-perfused *via* both carotid arteries with 0.1 M phosphate buffered saline (PBS, pH 7.4) for 5 min, followed by a fresh fixative solution containing 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) for 40 min. After perfusion, brains with attached pituitaries intact were removed, blocked, and 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 14 μm with a cryostat (Bright Instruments, Huntingdon, UK). Sections were mounted on microscope slides (Probe-on, Fisher Scientific, Minneapolis, MN, USA). Sections were stored desiccated at −80 °C until used for double *in situ* hybridization (ISH, *c-fos* mRNA)-immunohistochemistry (IHC, tyrosine hydroxylase; TH, and VIP).

Experiment 2: expression of 5-HT_{1A} and 5-HT_{2C} receptors in the hypothalamus during the turkey reproductive cycle

Thirty two Hybrid turkey hens were used. Hens were divided into four groups according to their reproductive status: (1) reproductively inactive, non-photostimulated (NPS) hens that were maintained under a 6L:18D lighting regime for at least 8 weeks, (2) laying (LAY) turkeys that had laid regularly up to the date of the experiment, (3) incubating (INC) birds that were nesting for at least 2 weeks, and (4) photorefractory (REF) birds that were molted and no longer laying or nesting despite the 15L:9D lighting schedule. The brains were fixed by pressure perfusion with 4% paraformaldehyde prior to sectioning in a cryostat and further processed for ISH is similar to that described in Experiment 1.

Riboprobes preparation

^{33}P-UTP-labeled antisense cRNA probes (*c-fos*, 5-HT_{1A} and 5-HT_{2C} receptors) were generated by an *in vitro* transcription reaction using a MAXIscript SP6 *In Vitro* Transcription Kit (Ambion Inc., Austin, TX, USA). The cRNA probes were purified by polyacrylamide gel electrophoresis. The specificity of the c-fos cRNA probe was previously verified by Northern blot analysis and ^{33}P-UTP-labeled antisense and sense cRNA probes (Al-Zailaie et al., 2006).

Specificity of 5-HT_{1A} and 5-HT_{2C} probes

To verify the specificity of 5-HT_{1A} and 5-HT_{2C} receptors expression, labeled and unlabeled mRNA probes were transcribed using a MEGAscript Kit (Ambion Inc.) The amount of mRNA corresponding to one million counts per minute of labeled probe was determined and hybridized overnight to a tissue section. A 100-fold excess of unlabeled probe was added to the hybridization buffer and used to hybridize an adjacent section. No expression was observed on tissue sections when labeled probe was hybridized in the presence of 100-fold excess of unlabeled probe.

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