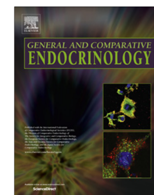
General and Comparative Endocrinology 204 (2014) 25–32

Contents lists available at ScienceDirect

General and Comparative Endocrinology

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



Regulation of gene expression of vasotocin and corticotropin-releasing hormone receptors in the avian anterior pituitary by corticosterone

Seong W. Kang *, Wayne J. Kuenzel

Department of Poultry Science, Center of Excellence for Poultry Science, University of Arkansas, Fayetteville, AR, United States

ARTICLE INFO

Article history:
Received 1 January 2014
Revised 21 March 2014
Accepted 14 April 2014
Available online 6 May 2014

Keywords:
Psychological stress
V1a receptor
V1b receptor
Chicken
HPA axis

ABSTRACT

The effect of chronic stress (CS) on gene expression of the chicken arginine vasotocin (AVT) and corticotropin-releasing hormone (CRH) receptors [VT2R, VT4R, CRH-R1, and CRH-R2] was examined by measuring receptor mRNA levels in the anterior pituitary gland of the chicken after chronic immobilization stress compared to acute stress (AS). Radioimmunoassay results showed that blood circulating corticosterone (CORT) levels in the CS group were significantly decreased compared to that of birds in the AS group ($P < 0.05$). The VT2R and CRH-R2 mRNA in CS birds were significantly decreased to that of controls. The VT4R mRNA was significantly decreased compared to controls in AC birds and was further decreased in the CS group compared to controls ($P < 0.05$). The CRH-R1 mRNA was significantly decreased in the AS birds compared to controls. However, there was no significant difference of CRH-R1 mRNA between acute stress and chronic stress birds. Using primary anterior pituitary cell cultures, the effect of exogenous CORT on VT/CRH receptor gene expression was examined. Receptor mRNA levels were measured after treatment of CORT followed by AVT/CRH administration. The CORT pretreatment resulted in a dose-dependent decrease of proopiomelanocortin heteronuclear RNA, a molecular marker of a stress-induced anterior pituitary. Without CORT pretreatment of anterior pituitary cell cultures, the VT2R, VT4R and CRH-R1mRNA levels were significantly increased within 15 min and then decreased at 1 h and 6 h by AVT/CRH administration ($P < 0.05$). Pretreatment of CORT in anterior pituitary cells induced a dose-dependent increase of VT2R, VT4R and CRH-R2 mRNA levels, and a significant decrease of CRH-R1 mRNA levels at only the high dose (10 ng/ml) of CORT ($P < 0.05$).Taken together, results suggest a modulatory role of CORT on the regulation of VT/CRH receptor gene expression in the avian anterior pituitary gland dependent upon CORT levels.

© 2014 Elsevier Inc. All rights reserved.

1. Introduction

Two neuropeptides, corticotropin-releasing hormone (CRH) and arginine vasopressin (AVP)/vasotocin (AVT: a non-mammalian homologue of AVP), are major regulators of the hypothalamic–pituitary–adrenal (HPA) axis during the stress response. The HPA axis adaptation with a reduction of CRH but a major increase of AVP has been shown during chronic stress (Antoni, 1993; Lightman, 2008; Mason et al., 2002; Murat et al., 2012). The effect of AVP, both alone and in combination with CRH, on adrenocorticotropic hormone (ACTH) release has been investigated in mammals (Antoni, 1986; Autelitano et al., 1989). The vasopressin V1b receptor (mammalian analog of avian vasotocin receptor subtype two: VT2R) is the most abundant mammalian vasopressin receptor in the anterior pituitary and facilitates release of ACTH during acute stress or chronic stress (Hernando et al., 2001; Ostrowski et al., 1992; Sugimoto et al., 1994). The release of CRH and AVP/AVT activates proopiomelanocortin (POMC) in the anterior pituitary ACTH-producing cells (corticotropes) through the CRH-R1 and the V1bR/VT2R and VT4R in chickens mediating the release of ACTH into peripheral blood where it targets receptors in the adrenal cortex causing the release of glucocorticoid hormones (Kuenzel et al., 2013; Lightman, 2008; Lundblad and Roberts, 1988; Mikhailova et al., 2008; Salem et al., 1970; Selvam et al., 2013).

In birds, VT2R, VT4R, CRH-R1 and CRH-R2 appear to be the major subtypes of VT/CRH receptors in the anterior pituitary based upon immunoreactivity of VT receptors in corticotropes and the relative expression levels of VT/CRH receptor subtypes (De Groef et al., 2003, 2004; Jurkevich et al., 2005, 2008; Kuenzel et al., 2013; Selvam et al., 2013). The VT2R appears to play a role in mediating the stimulatory effects of AVT on ACTH secretion from

* Corresponding author. Current address: Poultry Science Center, University of Arkansas, 1260 W. Maple Street, Fayetteville, AR 72701, United States. Fax: +1 479 575 7139.

E-mail addresses: kang_seong@hotmail.com, swkang@uark.edu (S.W. Kang).

http://dx.doi.org/10.1016/j.ygcen.2014.04.018
0016-6480/© 2014 Elsevier Inc. All rights reserved.

corticotropes and to a lesser extent on prolactin (PRL) secretion from lactotropes, based upon the presence of the immunoreactive VT2R in corticotropes and lactotropes, respectively (Cornett et al., 2003; Jurkevich et al., 2005, 2008; Kuenzel et al., 2013). In addition to the VT2R, the immunoreactive VT4R was found in corticotropes but not in lactotropes in bird (Selvam et al., 2013). Similar to mammals, CRH is a potent and specific stimulator of ACTH secretion by avian pituitary cells (Carsia et al., 1986) and simultaneous administration of AVT and CRH potentiates the release of corticosterone (CORT) in the blood circulation (Kuenzel et al., 2013; Mikhailova et al., 2007). This process is synergistic, because co-application of low amounts of AVP/AVT and CRH, which cannot elicit ACTH release on their own, results in a significant secretion of ACTH in mammals and CORT in birds (Baker et al., 1996; Gillies et al., 1982; Mikhailova et al., 2007). Repeated stimulation in mammals, however, with either peptide can cause reduced ACTH responsiveness or desensitization, both *in vivo* and *in vitro* (Mason et al., 2002). Recent studies suggest that the molecular interactions between V1b and CRH-R1 receptors in mammals and between VT2R and CRH-R1 receptors in chickens may play an important role in mediating the synergistic response in corticotropes (Cornett et al., 2013; Mikhailova et al., 2008; Murat et al., 2012).

The effect of glucocorticoid hormones on anterior pituitary POMC gene expression has been well documented (Autelitano et al., 1989; Eberwine et al., 1987). Rapid stimulation of POMC gene transcription was observed in mammalian and avian primary anterior pituitary cell cultures following treatment with CRH (Eberwine et al., 1987; Gagner and Drouin, 1987). Our recent studies indicate that AVT/CRH (1.0/0.1 nM) have an additive effect in stimulating POMC heteronuclear (hn) RNA expression in avian anterior pituitary cells (Jayanthi et al., 2014). Additionally, avian VT/CRH receptors in anterior pituitary gland differentially responded to acute immobilization stress (Kuenzel et al., 2013; Selvam et al., 2013). While events including transcription, processing, and receptor distribution of VP/VT and CRH receptors have been well described in both mammalian and avian species (Baertschi and Friedli, 1985; Johnstone et al., 2000; Kuenzel et al., 2013; Mikhailova et al., 2007; Selvam et al., 2013), the mechanisms, dynamics, and consequences of acute and chronic stress induced by CORT feedback inhibition on VP/VT and CRH receptor expression are largely unknown. The present study was performed to test the effects of chronic stress on VT/CRH receptor gene expression in the anterior pituitary gland of the chicken and the effects of exogenous corticosterone on VT/CRH receptor gene expression in primary anterior cells of the chicken.

2. Materials and methods

2.1. Animals and immobilization stress

One-day old male chicks were raised and fed a standard starter diet *ad libitum*. At two-weeks of age, birds were randomly assigned to four treatment groups ($n = 8$/treatment): acute control (AC), acute stress (AS), chronic control (CC) and chronic stress (CS). All birds were kept individually in cages under a 16 h light and 8 h dark lighting cycle (lights on 07:00 h) until 6 weeks old. Acute stress was induced via 1 h of immobilization, during which each bird was wrapped in a harness and restrained from standing or moving their upper bodies including their wings. Each bird had access to water as previously described (Jayanthi et al., 2014; Selvam et al., 2013). To minimize human presence and handling stress, sampling of blood from each bird was consistently and gently performed by the same person who had cared for the birds from day one. Birds designated as acute stressed birds were taken out from their cages and blood was sampled from the wing vein (brachial vein) using a 23 gauge heparinized needle and syringe before and after 1 h acute immobilization stress. Acute control birds were taken out of their cages, blood sampled, and returned to their cage. The process was repeated 1 h later. Birds chronically stressed were exposed to 1 h immobilization for 10 consecutive days from 6 weeks old. Blood was sampled on days 1, 4, 9, and 10 after 1 h stress. Regarding chronic control birds, blood was sampled on days 1, 4, 9 and 10 without a stress treatment. At the end of each 1 h period (acute treatment and controls), stress treatments and blood sample, anterior pituitary glands were collected and stored at −80 °C. All animals used in experiments were treated in accordance with protocols approved by the University of Arkansas Institutional Animal Care and Use Committee.

2.2. Radioimmunoassay (RIA) of plasma CORT

Plasma CORT levels were determined by RIA (Madison et al., 2008; Proudman and Opel, 1989). The primary antibody against CORT was purchased from Fitzgerald Inc. (Concord, MA, USA) while the secondary antibody and ^{125}I CORT tracer were purchased from MP Biomedicals Inc. (Orangeburg, NY, USA). The intra- and inter-assay coefficients of variation were 9% and 14%, respectively.

2.3. Primary anterior pituitary cell culture and CORT treatment before AVT/CRH administration

Primary anterior pituitary cells were obtained from 7 to 8 weeks old birds using a modified trypsin/neuraminidase procedure, as previously described (Fehrer et al., 1985; Jayanthi et al., 2014; Kang et al., 2002, 2004). Cultures were maintained at 39 °C in a humidified 5% CO_2/95% air incubator for 4 days. Cell viability (87–95%) was determined by trypan blue dye exclusion and quantified using a hemocytometer. In our previous study we established optimum doses of synergistic activation of POMC hn RNA by AVT/CRH treatment (Jayanthi et al., 2014). Therefore, in this *in vitro* study, dispersed anterior pituitary cells (0.5×10^6) were treated with combinations of AVT(1.0 nM; Bachem Americas Inc. Torrance, CA, USA) and CRH (0.1 nM; Bachem Americas Inc., Torrance, CA, USA) for 15 min, 1 h, and 6 h as previously described (Jayanthi et al., 2014), or were pretreated with different concentrations of CORT (0, 10, 100 pg/ml, 1, 10 ng/ml; Sigma–Aldrich, USA) 30 min before AVT/CRH treatment (1.0/0.1 nM). At the conclusion of each treatment, anterior pituitary cells were collected by centrifugation, washed with 2 ml of phosphate-buffered saline (PBS), and dissolved in 1 ml Trizol® reagent (Life Technologies, Palo Alto, CA, USA) and frozen at −80 °C. The *in vitro* primary cell culture study was repeated two times, and in each cell culture measurements of gene expression were done in triplicate and repeated two times for consistency.

2.4. RNA isolation and two-step real-time quantitative RT-PCR

Total RNA was extracted from quick-frozen anterior pituitary samples or cultures of primary anterior pituitary cells using TRIzol® reagent (Invitrogen Life Technologies, Palo Alto, CA, USA) following the DNase I treatment and purification of total RNA by RNeasy mini kit (Qiagen, Valencia, CA, USA). The RNA quality and quantity were determined using agarose gel electrophoresis and NanoDrop 1000 (Thermo Scientific, Wilmington, DE, USA). Three micrograms of total RNA from fresh-frozen anterior pituitary glands or primary anterior pituitary cells were converted into cDNA with oligo $(dT)_{16}$ primer and SuperScript III reverse transcriptase (Invitrogen Grand Island, NY, USA), as previously described (Jayanthi et al., 2014; Kang et al., 2007, 2010).The PCR primer pairs for POMC hnRNA, AVT and CRH receptor genes were previously reported (Jayanthi et al., 2014; Kuenzel et al., 2013; Selvam et al., 2013). The best primer pairs were initially selected

Download English Version:

https://daneshyari.com/en/article/2800171

Download Persian Version:

https://daneshyari.com/article/2800171

Daneshyari.com