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Chicken domestication changes expression of stress-related genes in brain, pituitary and adrenals

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ABSTRACT

Domesticated species have an attenuated behavioral and physiological stress response compared to their wild counterparts, but the genetic mechanisms underlying this change are not fully understood. We investigated gene expression of a panel of stress response-related genes in five tissues known for their involvement in the stress response: hippocampus, hypothalamus, pituitary, adrenal glands and liver of domesticated White Leghorn chickens and compared it with the wild ancestor of all domesticated breeds, the Red Junglefowl. Gene expression was measured both at baseline and after 45 min of restraint stress. Most of the changes in gene expression related to stress were similar to mammals, with an upregulation of genes such as *FKBP5*, *C-FOS* and *EGR1* in hippocampus and hypothalamus and *StAR*, *MC2R* and *TH* in adrenal glands. We also found a decrease in the expression of *CRHR1* in the pituitary of chickens after stress, which could be involved in negative feedback regulation of the stress response. Furthermore, we observed a downregulation of *EGR1* and C-*FOS* in the pituitary following stress, which could be a potential link between stress and its effects on reproduction and growth in chickens.

We also found changes in the expression of important genes between breeds such as *GR* in the hypothalamus, *POMC* and *PC1* in the pituitary and *CYP11A1* and *HSD3B2* in the adrenal glands. These results suggest that the domesticated White Leghorn may have a higher capacity for negative feedback of the HPA axis, a lower capacity for synthesis of ACTH in the pituitary and a reduced synthesis rate of corticosterone in the adrenal glands compared to Red Junglefowl. All of these findings could explain the attenuated stress response in the domesticated birds.

1. Introduction

A number of animal species have adapted to living in the captive environments provided by humans during domestication, allowing the individuals to tolerate proximity to humans, and to live in crowded and confined conditions (Price, 1999). Direct selection by humans, natural selection in the new environment and genetic drift have led to a suite of traits that are commonly associated with domestication, the domesticated phenotype. This includes changes in morphology, physiology and behavior (Price, 1999, 2002) and modifications in the response to stressful stimuli. Stress can be defined as the individual's response to real or perceived threats to homeostasis (McEwen, 2000, 2007). The key regulator of the physiological stress response is the hypothalamic-pituitary-adrenal (HPA) axis, with the adrenal glands secreting glucocorticoids into the blood stream.

Glucocorticoids have a wide variety of effects depending on the target tissue, including glycogen breakdown and gluconeogenesis (Exton, 1978; Munk et al., 1984; Coderre et al., 1991; Myers et al., 2014). Their overall function is to shift resource allocation to promote immediate survival, for instance counteracting blood loss and mobilizing energy, while suppressing body functions that are not crucial for immediate survival, such as reproduction, immune system and digestion (Herman et al., 2016). While a stress-induced release of glucocorticoids is beneficial in a short-term challenge, long-term exposure to stress may be harmful to the individual being associated with susceptibility to several diseases, and lowered reproductive ability (De Kloet et al., 2005; Chrousos, 2009). The HPA axis is dependent on a negative feedback system, in which binding of glucocorticoids to glucocorticoid receptors at several levels within the axis can inhibit its activity (De Kloet et al., 2005; Vandenborne et al., 2005; Chrousos, 2009; Keller-Wood, 2011).

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The HPA axis has been modified in several different domesticated species in such a way that they have a lower physiological response to acute stress (Weiler et al., 1998; Künzl and Sachser, 1999; Gulevich et al., 2004; Trut et al., 2009; Plyusnina et al., 2011; Soleimani et al., 2011). This modulation may be achieved through modified activity of any of the many proteins involved. A multitude of genes affects this in various tissues, coding for hormones or hormone precursors, receptors, cleaving enzymes, transcription factors and cofactors (Cullinan et al., 1995a; Liu et al., 2013). Any modification in the expression of these genes or the translation of their mRNA to proteins, may lead to changes in the activity of the HPA axis, and highlights possible candidates for explaining domestication effects of the stress response. Upon exposure to an acute stressor, corticotropin-releasing hormone (CRH) and arginine vasopressin (AVP) (in mammals) or arginine vasotocin (in birds) are secreted from the paraventricular nucleus (PVN) of the hypothalamus and transported through the portal vessel to the pituitary (Blas, 2015). Here, CRH stimulates the secretion of adrenocorticotropic hormone (ACTH) into the general circulation. When ACTH reaches the adrenal glands, it initiates the production and release of glucocorticoids, i.e. cortisol, in most mammals and corticosterone in birds and rodents, from the adrenal cortex (Carsia, 2015; Herman et al., 2016).

Additionally, changes in the metabolism of glucocorticoids, mainly taking place in the liver, may also affect the function of the HPA axis (Keller-Wood, 2011). Although there may have been direct selection on stress response and tolerance during domestication, the reduced HPA axis activity observed in domesticated animals may also be partly explained as side effects of selection for other traits due to linkage and pleiotropy (Rauw et al., 1998; Schütz et al., 2004).

The HPA axis in mammals is generally well studied, and much detail is available concerning which genes are involved and how they are controlled (Payne and Hales, 2004; Ellis et al., 2006; Ulrich-Lai and Herman, 2009; Mormede et al., 2011; Herman et al., 2016). However, less is known about the details of the stress response in birds.

Comparing Red Junglefowl (RJF) and domesticated chickens represents an excellent platform for investigating effects of domestication on traits such as the stress response. Domestication of the chicken is thought to have taken place approximately 8000 years ago in South East Asia from a common ancestor, the Red Junglefowl (Tixier-Boichard et al., 2011; Storey et al., 2012; Xiang et al., 2014), still present in its natural habitats in South East Asia.

Investigations of the stress response of these two breeds have previously shown a more fearful behavior, as well as a more pronounced corticosterone increase after acute stress, in RJF (Ericsson et al., 2014; Fallahsharoudi et al., 2015). However, RJF also appear to return to baseline faster than WL, both in terms of behavior and hormonal levels (Ericsson et al., 2014).

Thus, the aim of this study was to assess the effects of domestication on the stress response by monitoring the expression of selected genes involved in the activation and modulation of the HPA axis in five tissues known to be involved in the response in both the ancestral Red Junglefowl and the domesticated White Leghorn breed.

2. Methods

2.1. Overview of methods

In this project, we used male Red Junglefowl (RJF) and White Leghorns (WL) to investigate the changes in gene expression to an acute stress treatment. At 7 weeks of age, the animals in the stress group were exposed to 45 min of restraint stress. They were then culled, and tissue from hippocampus, hypothalamus, pituitary, adrenals and liver were collected. A group of baseline birds (not exposed to restraint stress) were also culled and sampled for the same tissues. Gene expression analysis with quantitative PCR was then performed on all tissues to compare baseline (unstressed animals) against stress treatment.

Our selection of genes was based both on literature reviews, mainly from rodents, and our previous experiments on chickens. Specifically, in the brain, we focused on genes involved in stimulation and negative feedback of the HPA axis. In the pituitary, we mainly chose genes involved in translating CRH signals to ACTH release. Most of the selected genes in the adrenal glands are involved in steroidogenesis and sympathoadrenal activity, whereas in the liver, we measured genes coding for the corticosterone binding globulin and a gene involved in the metabolism of glucocorticoids.

2.2. Ethical statement

All experimental protocols were approved by Linköping Council for Ethical Licensing of Animal Experiments, ethical permit no 50-13. Experiments were conducted in accordance with the approved guidelines.

2.3. Animals and housing

We studied one population of domesticated WL and one population of ancestral RJF. The WL population in this experiment (SLU13) was the progeny of an outbred line selected for egg mass and developed for research purposes. The studied RJF population originated from a wild population in Thailand (see (Schütz et al., 2001) for details about the origin of used populations in the experiment). All animals were hatched in our facility and were kept under 12 h light and dark periods with ad libitum access to food and water. The breeds were kept separately in similarly sized (2 m $\times$ 2 m) enclosures and similar conditions until they were 6 weeks old when the experiment was conducted. The birds were hatched and kept in groups of around 70 animals, until they were 3 weeks, then they were blood sampled and sex determined. The male birds were then kept in breed-separated pens in groups of around 24 animals.

2.4. Sex determination

At the age of three weeks, the chickens were blood sampled from the brachial vein for sex determination. Genomic DNA was extracted from the blood samples according to standard protocols, and sex determination was performed using qPCR based on the method described in Clinton et al. (2001).

2.5. Tissue collection

Eight animals from each breed were culled and sampled without going through the stress procedure, whereas eight animals from each breed were culled after 45 min of stress, amounting to a total of 32 birds. Culling was performed by decapitation, and dissection took place immediately after. The top of the skull was opened, and the whole brain was removed. The hippocampal and parahippocampal areas were dissected out from both hemispheres by making a small incision close to the rostral part of the lateral ventricle. Tweezers were then used to carefully pull loose the tissue dorsally and medially to the lateral ventricle, corresponding to the avian hippocampus and parahippocampal area (Puelles, 2007).

Further dissection was performed to dissect a part of the brain enriched in thalamus/hypothalamus from the diencephalon. The