General and Comparative Endocrinology 221 (2015) 278–283

Contents lists available at ScienceDirect

General and Comparative Endocrinology

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

Minireview

Regulation of the avian central melanocortin system and the role of leptin

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ARTICLE INFO

Article history:
Available online 9 January 2015

Keywords:
AGRP
POMC
AMPK
Leptin
Arcuate nucleus
Chicken

ABSTRACT

The avian central melanocortin system is well conserved between birds and mammals in terms of the component genes, the localisation of their expression in the hypothalamic arcuate nucleus, the effects on feeding behaviour of their encoded peptides and the sensitivity of agouti-related protein (*AGRP*) and pro-opiomelanocortin (*POMC*) gene expression to changes in energy status. Our recent research has demonstrated that *AGRP* gene expression precisely differentiates between broiler breeder hens with different histories of chronic food restriction and refeeding. We have also shown that the sensitivity of *AGRP* gene expression to loss of energy stores is maintained even when food intake has been voluntarily reduced in chickens during incubation and in response to a stressor. However, the similarity between birds and mammals does not appear to extend to the way *AGRP* and *POMC* gene expression are regulated. In particular, the preliminary evidence from the discovery of the first avian leptin (*LEP*) genes suggests that LEP is more pleiotropic in birds and may not even be involved in regulating energy balance. Similarly, ghrelin exerts inhibitory, rather than stimulatory, effects on food intake. The fact that the importance of these prominent long-term regulators of *AGRP* and *POMC* expression in mammals appears diminished in birds suggests that the balance of regulatory inputs in birds may have shifted to more short-term influences such as the tone of cholecystokinin (CCK) signalling. This is likely to be related to the different metabolic fuelling required to support flight.

1. Introduction

The avian melanocortin system genes were all cloned and characterised 14 years ago. They comprise five melanocortin receptors (Takeuchi et al., 1996, 1998; Takeuchi and Takahashi, 1998, 1999), and the pro-opiomelanocortin (*POMC*) and agouti-related protein (*AGRP*) genes encoding their endogenous agonists and antagonists, respectively (Berghman et al., 1998; Takeuchi et al., 1999, 2000). The central melanocortin system shows evolutionarily functional and neuroanatomical conservation in comparison with mammals. Thus, melanocortin signalling is regulated by opposing, agonistic and antagonistic actions of endogenous α-melanocyte-stimulating hormone (α-MSH) and agouti-related protein (AGRP) that, respectively, increase and decrease food intake after central injection (Tachibana et al., 2001; Strader and Buntin, 2003; Strader et al., 2003). Also, the *AGRP* and *POMC* genes are expressed in the arcuate nucleus of the hypothalamus where *AGRP* mRNA is co-expressed with neuropeptide Y (*NPY*) mRNA in individual arcuate nucleus neurons (Boswell et al., 2002). In this mini-review we focus on the regulation of the *AGRP* and *POMC* genes in birds with emphasis on our recent work on chronic food restriction and natural models of voluntarily reduced food intake. We also consider the implications of the identification and preliminary characterisation in 2014 of the first avian leptin (*LEP*) genes.

2. Effects of manipulating energy status

The functional evolutionary conservation of the avian central melanocortin system extends to its response to manipulation of energy status. Most investigations have focused on short-term food deprivation, which increases *AGRP* mRNA (Phillips-Singh et al., 2003; Higgins et al., 2010; Song et al., 2012). Changes in mRNA appear to reflect parallel changes in peptide because food deprivation increased the number of AGRP-immunoreactive cells in the arcuate nucleus of the ring dove (*Streptopelia risoria*) which were also elevated during the parental phase of the breeding cycle when the birds are in negative energy balance (Strader and Buntin, 2003; Strader et al., 2003). For *POMC*, Higgins et al. (2010) observed a significant decrease in mRNA after food deprivation and two studies have reported a non-significant decrease (Phillips-Singh et al.,

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http://dx.doi.org/10.1016/j.ygcen.2014.12.009
0016-6480/

2003; Song et al., 2012). Thus the changes in *AGRP* and *POMC* expression during fasting are in the direction predicted from the behavioural effects of their encoded peptides as part of a coordinated counter-regulatory response of hypothalamic appetite control peptides to restore lost energy stores (Phillips-Singh et al., 2003). Higgins et al. (2010) used microarray analysis to explore patterns of gene expression in the hypothalamus of domestic chicks in relation to food deprivation. Pathway analysis software predicted that *POMC* lies within a network of six interrelated differentially-expressed genes and experimental evidence for the existence of the network was provided from *in vitro* experiments. In addition to *POMC*, the network contained genes encoding the neuropeptide relaxin-3, the neuropeptide receptors NPY5R and somatostatin receptor 5, and the β2 adrenergic and metabotrophic glutamate receptor 8 neurotransmitter receptors (Fig. 1). *POMC* was the only gene downregulated by fasting while the others in the network were upregulated. This suggests a role for these pathways in the control of appetite within the hypothalamus. Although elements of the network such as the *POMC* and *NPY5R* genes are involved in the response to food deprivation in mammals, the network as a whole was uniquely observed in the Higgins et al. (2010) study. There is also suggestive evidence for a role of hypothalamic energy sensing pathways in regulating central melanocortin signalling. Proszkowiec-Weglarz et al. (2006) observed strong immunostaining for the energy sensor AMP-activated protein kinase (AMPK) in the arcuate nucleus and Song et al. (2012) reported changes in AMPK activity indicated by phosphorylation during food deprivation and refeeding that paralleled changes in *AGRP* gene expression.

In comparison to food deprivation, the effects of chronic food restriction on the central melanocortin system have received less attention. We recently measured *AGRP* and *POMC* gene expression in broiler breeder hens subjected to different levels of chronic food restriction and refeeding (Dunn et al., 2013a). Food restriction for six weeks at a level routinely used in the poultry industry to improve reproductive performance strongly stimulated *AGRP* gene expression. We also made comparisons between hens of the same body mass that had experienced different feeding histories. Thus, we were able to distinguish in terms of significant changes in *AGRP* mRNA between birds killed at the same body mass that had been either been maintained on an intermediate level of food restriction or more severely restricted and then refed for two weeks. We also observed a difference between restricted birds compared to restricted hens that had been allowed to refeed for two days, with refeeding causing a pronounced decrease in *AGRP* mRNA. Overall we found that *AGRP* mRNA provided an integrated and sensitive measure of feeding history and has potential as an objective measure of hunger in animal welfare research. As has been observed in food deprivation studies, there appears to be a tendency for *POMC* gene expression to be decreased during food restriction. Hen et al. (2006) reported a significant decrease in *POMC* mRNA after seven days' food restriction in broiler and layer chickens. However a significant change in *POMC* expression does not appear to be as consistent as that for *AGRP* mRNA (Dunn et al., 2013a) suggesting that increased *AGRP* expression is of greater relative importance in the compensatory response to food restriction.

3. Seasonal and stress-induced changes in body mass

Seasonal cycles of food intake and body mass have been well studied in birds, particularly in relation to migration, but little is known about the regulatory role of the central melanocortin system (Cornelius et al., 2013). Experiments in white-crowned sparrows (*Zonotrichia leucophrys gambelii*) have suggested that increases in body mass during the migratory period are associated with a regulated change in the level around which body mass is defended, a phenomenon known as a 'sliding set point' or rheostasis (King et al., 1963; Mrosovsky, 1990). One hypothesis is that seasonal changes in appetite and body weight are driven by an altered basal expression of *AGRP* and *POMC* in a direction reflecting the behavioural effects of the peptides. For example, to generate a seasonal body mass increase, the expression of *AGRP* and *POMC* would be respectively increased and decreased. However preliminary evidence from experiments in the European quail (*Coturnix coturnix*) suggested that *AGRP* and *POMC* gene expression were unchanged at a time when food intake and body mass were increasing during the migratory period (Cornelius et al., 2013). These findings are reminiscent of those from studies of seasonal mammals where the rheostatic mechanisms appear to lie in brain areas outside the arcuate nucleus (Ebling, 2014).

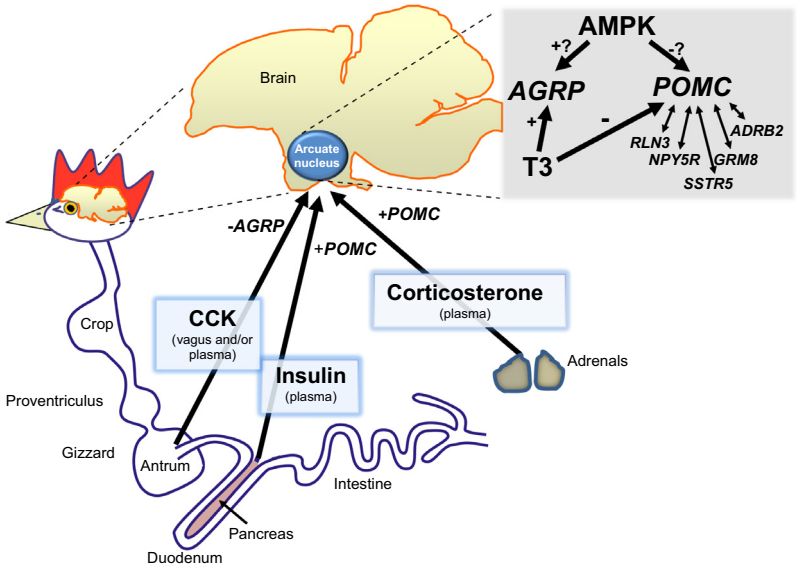

Fig. 1. Summary of current knowledge of regulatory inputs (positive and negative influences are indicated) on agouti-related protein (*AGRP*) and pro-opiomelanocortin (*POMC*) gene expression in the arcuate nucleus of the avian hypothalamus. Signalling within the hypothalamus is shown in the box. Italicised symbols indicate interactions at the level of gene expression. Abbreviations: *ADRB2* – β2 adrenergic receptor; AMPK – AMP-activated protein kinase; *GRM8* – metabotrophic glutamate receptor 8; *NPY5R* – neuropeptide Y receptor Y5; *RLN3* – relaxin-3; *SSTR5* – somatostatin receptor 5.