RESEARCH HIGHLIGHT

Orexin regulates mitochondrial dynamics in avian muscle

Elizabeth Greene, Kentu Lassiter, Walter Bottje, Sami Dridi

Center of Excellence for Poultry Science, University of Arkansas, Fayetteville, AR 72701, USA

Correspondence: Sami Dridi
E-mail: dridi@uark.edu
Received: February 11, 2015
Published online: April 02, 2015

The growing obesity epidemic has sparked numerous studies on the identification of molecular signatures that regulate energy homeostasis using different experimental animal models. Orexin, which acts via two G-protein coupled receptors, orexin receptor 1 and 2, has been originally identified as feeding-related hypothalamic neuropeptide that regulate energy balance in mammals. Recently, using chicken, non-mammalian species that are characteristically hyperglycemic and prone to obesity, we made a breakthrough by identifying the orexin system in avian muscle and unraveling its effect on mitochondrial dynamics and function. Therefore, understanding orexin signaling and function may help to identify novel therapeutic opportunities for treating metabolic disorders related to mitochondrial dysfunction.

Keywords: Orexin; avian species; energy homeostasis; mitochondrial dynamics

To cite this article: Elizabeth Greene, *et al*. Orexin regulates mitochondrial dynamics in avian muscle. Receptor Clin Invest 2015; 2: e632. doi: 10.14800/rci.632.

Orexin, also named hypocretin, was discovered in 1998 by orphan receptor technologies [1,2]. Prepro-orexin is enzymatically cleaved in two mature peptides, orexin A and orexin B which are 33- and 28-amino-acid peptides. Orexins signal through two serpentine G-protein coupled receptors, orexin receptor 1 and 2 (ORXR1/2) which contain seven putative transmembrane helices [2]. In mammals, ORXR1 preferentially binds orexin-A, whereas ORXR2 binds both peptides with similar affinity [2]. Orexins control several physiological processes in mammals including food and water intake [3], wakefulness [4], circadian clock [5], glucose homeostasis [6], lipid metabolism [7], and neuroendocrine response to stress [8]. In avian (non-mammalian) species, however, the role of orexin is not well defined.

Compared with mammals, chickens are characteristically hyperglycemic with their blood glucose levels averaging three times that found in humans [9]. Genetic selection for accelerated growth rate driven by economic demands has resulted in hyperphagic broiler chickens that are prone to obesity. Broiler chickens ravenously consume approximately 4.1 kg of feed to achieve a 40-fold increase in body weight arising from breast muscle and abdominal fat during a period of 42 days [9]. Chickens are insulin resistant and lacking glucose transporter GLUT4 [10]. They require higher doses (more than four times compared to mammals) of insulin to achieve hypoglycemia [11]. As in human, chicken liver, not adipose tissue, is the primary site for lipogenesis. However, and in contrast to human, chickens do not have brown adipose tissue (BAT). These distinctive hallmarks make chicken an interesting animal model to study the role and mode of orexin action in energy homeostasis regulation.

We recently found that orexin and its related receptor ORXR1/2 (mRNA and protein) are expressed in avian muscle tissue and cells [12]. Interestingly, we detected orexin protein not only in avian muscle cell lysates, but also in the medium with a steadily accumulation over a 48h-period indicating that orexin is a secretory protein. This hypothesis was supported by the absence of orexin expression in the medium and increased intracellular expression following the administration of brefeldin A, the inhibitor of translocation

of secretory proteins from the endoplasmic reticulum to the Golgi apparatus. The first 33 amino acids of prepro-orexin exhibit characteristics of a secretory signal sequence and orexin has been found in the circulation, however it is not known yet what portion of orexin comes from the muscle and what portion comes from other organs.

Treating the avian muscle cells with human recombinant orexin A or B (hr-OrxA/B) differentially regulated the expression of orexin and ORXR1/2. Indeed, hr-OrxA increased the expression of ORX and ORXR1 but not ORXR2, however hr-OrxB decreased ORX and ORXR2 and increased ORXR1 mRNA and protein levels. The mechanism underlying this divergent effect is not known at this time, but it might be related to the different structure of hr-OrxA and B (presence of disulfide bonds in orexin A and not in orexin B), their binding affinity, and/or to the function of the cytoplasmic tails and the desensitization of ORXR1/2 [12]. In line with these divergent effects, orexins differentially regulated mitochondrial biogenesis [mtDNA, mt single-stranded DNA binding protein 1 (mtSSBP1), oxphos complex IV subunit I (CoxIV), and Cox5a]- and function [uncoupling protein (UCP), adenosine nucleotide translocator (ANT), nuclear respiratory factor (NRF1), nuclear sarcoma viral oncogene homolog (Ski), peroxisome proliferator-activated receptor alpha/beta (PPARα/β), PPARδ coactivator 1 alpha/beta (PGC-1α/β) and forkhead box protein O1 (FoxO1)]-related genes and thereby mitochondrial bioenergetics (ATP synthesis, proton leak and reserve capacity). Interestingly, orexins also differentially regulated mitochondrial fusion- and fission-related genes. While hr-OrxB increased mitofusion, as indicated by the up-regulation of mitofusin 2 (MFN2), optic atrophy (OPA1) and OMA1 zinc metallopeptidase (OMA1) gene expression, hr-OrxA promoted mitofission as reflected in the increased expression of mitochondrial fission process (MTFP1), dynamin-related protein 1 (DNM1) and mitochondrial fission regulator 1 (MTFR1) genes [8].

Mitochondria are dynamic organelles that constantly fuse and divide, and an imbalance of these two processes dramatically alters mitochondrial morphology and function. Skeletal muscle mitochondria function is nearly linked with insulin resistance and mitochondrial number and size are declined in the skeletal muscle of obese and type 2 diabetic patients compared with lean healthy subjects [13]. Furthermore, a down-regulation of MFN2 expression accompanied with 25% reduction in the muscle mitochondrial network was observed in obese Zucker rats [14].

Taken together, we [12] made a breakthrough by identifying orexin system expression in chicken muscle and its pivotal role in mitochondrial function and dynamics. Because a lack of BAT, the chicken muscle is the main site for thermogenesis and because mitochondrial functions are involved in many (patho)-physiological processes, our findings open new perspectives on the role of orexin in muscle energy metabolism. Further studies dissecting the role of orexin in chicken muscle development, myogenesis, insulin sensitivity and glucose uptake are needed. Insights into these molecular mechanisms could help not only to develop new therapeutic strategies in molecular medicine but also to improve animal health and feed efficiency.

Conflict of interests

The authors declare that there is no conflict of interests.

Acknowledgements

This work was supported by startup funds from the State of Arkansas, ABI (FY2015) and ABI equipment grant (FY2014) from Arkansas Bioscience Institute to S.D. and ABI (FY2012-2015) to W.B.

References

1. L de Lecea, Kilduff TS, Peyron C, Gao X, Foye PE, Danielson PE, *et al*. The hypocretins: hypothalamus-specific peptides with neuroexcitatory activity. Proc Natl Acad Sci U S A 1998; 95: 322-327.
2. T Sakurai, Amemiya A, Ishii M, Matsuzaki I, Chemelli RM, Tanaka H, *et al*.Orexins and orexin receptors: a family of hypothalamic neuropeptides and G protein-coupled receptors that regulate feeding behavior. Cell 1998; 92: 573-585.
3. T Sakurai.Orexins and orexin receptors: implication in feeding behavior. Regul Pept 1999; 85: 25-30.
4. RM Chemelli, Willie JT, Sinton CM, Elmquist JK, Scammell T, Lee C, *et al*. Narcolepsy in orexin knockout mice: molecular genetics of sleep regulation. Cell 1999; 98: 437-451.
5. MD Belle, Hughes AT, Bechtold DA, Cunningham P, Pierucci M, Burdakov D, *et al*.Acute suppressive and long-term phase modulation actions of orexin on the mammalian circadian clock. J Neurosci 2014; 34: 3607-3621.
6. H Tsuneki, Wada T, Sasaoka T.Role of orexin in the regulation of glucose homeostasis. Acta Physiol (Oxf) 2010; 198: 335-348.
7. M Skrzypski, T TL, Kaczmarek P, Pruszynska-Oszmalek E, Pietrzak P, Szczepankiewicz D, *et al*. Orexin A stimulates glucose uptake, lipid accumulation and adiponectin secretion from 3T3-L1 adipocytes and isolated primary rat adipocytes. Diabetologia 2011; 54: 1841-1852.
8. WK Samson, Bagley SL, Ferguson AV, White MM.Hypocretin/orexin type 1 receptor in brain: role in cardiovascular control and the neuroendocrine response to immobilization stress. Am J Physiol Regul Integr Comp Physiol 2007; 292: R382-387.
9. SM Krzysik-Walker, Ocon-Grove OM, Maddineni SR, Hendricks

GL, 3rd, Ramachandran R.Is visfatin an adipokine or myokine? Evidence for greater visfatin expression in skeletal muscle than visceral fat in chickens. Endocrinology 2008; 149: 1543-1550.

10. Y Seki, Sato K, Kono T, Abe H, Akiba Y.Broiler chickens (Ross strain) lack insulin-responsive glucose transporter GLUT4 and have GLUT8 cDNA. Gen Comp Endocrinol 2003; 133: 80-87.
11. Y Akiba, Chida Y, Takahashi T, Ohtomo Y, Sato K, Takahashi K.Persistent hypoglycemia induced by continuous insulin infusion in broiler chickens. Br Poult Sci 1999; 40: 701-705.
12. K Lassiter, Greene E, Piekarski A, Faulkner OB, Hargis BM, Bottje W, *et al*. Orexin system is expressed in avian muscle cells and regulates mitochondrial dynamics. Am J Physiol Regul Integr Comp Physiol 2015; 308: R173-187.
13. DE Kelley, He J, Menshikova EV, Ritov VB.Dysfunction of mitochondria in human skeletal muscle in type 2 diabetes. Diabetes 2002; 51: 2944-2950.
14. D Bach, Pich S, Soriano FX, Vega N, Baumgartner B, Oriola J, *et al*. Mitofusin-2 determines mitochondrial network architecture and mitochondrial metabolism. A novel regulatory mechanism altered in obesity. J Biol Chem 2003; 278: 17190-17197.