Identification of Genuine/Authentic Avian Leptin: Some Answers and More Questions

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The dogmatic adherence to "what is good for the goose is good for the gander" appears to pervade avian endocrinology research when it comes to vertebrate evolution of hormones and their physiological roles in birds. There appears to be a general acceptance that specific hormones, their cognate receptors, and their regulatory functions identified in other vertebrates should be present and serve the same function in birds. This has been largely the case for leptin (LEP) originally identified in mammals (1–5), which has been vigorously sought for more than a decade in birds (6–10), and also more recently for kisspeptin (11, 12). But should we expect to find the same hormones and functions in birds as occurs in other vertebrates? In order for early reptilian bird ancestors to take to the air, phenomenal evolutionary changes in their physiology were required and presumably drove changes in the use of existing hormones and cognate receptors, their modification, or their abandonment. Yet many scientists take the view that major physiological systems present in tetrapods and fish should be present in birds and serve the same function and use of the same hormone/receptor regulators.

This is clearly not the case for some major systems, such as the genetics of sexual determination in birds and the high set point for body temperature (40°C), and for some major hormone regulators, such as the neuroendocrinology of the regulation of reproduction. The latter is characterized by the total excision of kisspeptin and kisspeptin receptor genes from the avian genome but its complete conservation as an essential regulator of reproduction in all other vertebrates from fish to mammals (11, 12). At the same time, a potent negative regulator of reproduction in birds, gonadotropin-inhibitory hormone, has evolved and appears to play a more prominent role in avian reproduction than in other vertebrate classes (13).

It is therefore perhaps not altogether surprising that the LEP system of metabolic regulation and appetite regulation might not exist/operate in birds. Research over more than a decade had suggested that this is the case (6–10). This was contradicted by several reports of the identification of a *LEP* gene with high identity with the mammalian *LEP* gene (14, 15). However, these putative *LEP* genes had greater sequence identity with mammalian *LEP* genes than with any other vertebrate sequences, strongly suggesting contamination with mammalian *LEP* gene as pointed out in the current articles.

Has an Authentic/Genuine Avian "LEP" Now Been Found?

In the first of 2 articles in the current issue of Endocrinology, "Discovery of a novel functional Leptin protein (LEP) in zebra finches: evidence for the existence of an authentic avian leptin gene predominantly expressed in the brain and pituitary," Huang et al (16) identified and characterized a putative LEP gene (*zbLEP*) encoding a 172-amino acid precursor in zebra finches. This gene has 26% and 29% amino acid sequence identity with human and mouse LEP, respectively. Synteny analysis showed that *zbLEP* is orthologous to mammalian *LEP* gene.

In the second article, "Discovery and characterization of the first genuine avian leptin gene in the rock dove (*Columba livia*)," Friedman-Einat et al (17) discovered the same gene in the rock dove encoding a 181-amino acid orthologous precursor with 30% identity to the human

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Abbreviations: LEP, leptin; LEPR, LEP receptor.

ortholog. These 2 important discoveries indicate that the previous reports and database depositions of *LEP* genes in chickens, ducks, and turkeys with more than 90% identity with mammalian LEP are clearly due to contamination with mammalian LEP as alluded to above. This author (R.P.M.) was once presented in his laboratory with a sequence of cloned chicken GnRH that had greater identity with human than the mouse GnRH gene, which immediately had alarm bells ringing. Researchers need to be alerted to the fact that species identities of hormones and receptors that do not conform to phylogenetic relationships should be regarded with skepticism and not committed to publication or deposited in databases without rigorous elimination of the possibility of contamination.

Interestingly, both studies in this month's Endocrinology found that LEP is not expressed in adipose tissue, which suggests that it does not act as an adipocyte-derived signal to control energy balance. This result calls into question whether the gene that has been discovered should be regarded as a *LEP* gene at all and perhaps referred to as LEP-like hormone (see commentary below). There is also a disturbing difference in tissue expression of LEP observed by the 2 studies. Huang et al (16) find that LEP expression is almost exclusively in the pituitary of the zebra finch, whereas Friedman-Einat et al (17) record predominant expression in the liver and in the gonads of the rock dove and poor expression in pituitary. If the identified gene is an important physiological regulator, how can its major expression be in such different tissues in different bird species? Indeed, this brings into question the physiological significance of LEP in birds or suggests that there is considerable plasticity in its use that is not apparent in other vertebrates in which LEPs, with clear relationship to the mammalian counterpart, have been identified (18–21). Plasticity in use of hormone structures for different functions is exemplified by GnRHs (22) but so is the silencing of genes as has occurred with GnRH II and its cognate receptor that may have been selected for during domestication (23).

Both articles document that LEP receptor (LEPR) expression predominates in the pituitary (not the hypothalamus) and is also expressed in the gonads. Friedman-Einat et al (17) note that there is LEP activity in the circulation, suggesting that, if the differential expression patterns reported by the 2 studies can be reconciled, one could put forward the concept that LEP is a pituitary hormone that acts on the gonads but is also an autocrine stimulator of pituitary hormones in birds. In keeping with this, previous studies have described direct actions of LEP on LH and FSH as well as GH secretion. However, it is wise to be cautious on such interpretations and take a more parsimonious view, at least until more extensive studies have been conducted.

Although the avian LEP does not appear to have a LEP role, it is clearly active at the avian LEPR. Huang et al (16) observed, using a pAH32 luciferase reporter system and Western blotting of STAT3 phosphorylation analysis, that the recombinant finch protein potently (nanomolar range) activated finch and chicken LEPRs expressed in HEK293 cells. This observation is encouraging but also somewhat surprising, because the authors note that the avian ortholog has about 10 amino acids missing in the connecting loop compared with mammalian LEP and that these are essential for receptor activation but not binding.

Should the Newly Identified Avian LEP Ortholog Sequences Be Regarded as LEP?

The identification and naming of LEP was based on the pursuit of a humoral factor produced by adipose tissue that regulated appetite. The novel gene identified by the research groups in bird species is not produced by adipose tissue, LEPR is expressed at highest levels outside the hypothalamus, and LEP does not appear to play a role in appetite regulation in birds. Thus, should it be referred to as LEP in birds? Or would it be wiser to refer to the new gene as a LEP ortholog (ortho-LEP) and name the protein (with only 30% identity) "LEP-like" or dub it with a new name that reflects its function, once identified, so as not to confuse by implying LEP function? After all, IGF-I has more identity (48%) to insulin than does avian and mammalian LEP.

Conclusion/Summary

In summary, 2 articles describe the identification of novel avian orthologs of tetrapod LEP. However, these have low homology with the mammalian hormone, and their tissue distribution, as well as that of its receptor, is not compatible with an adipocyte signaler to appetite centers in the hypothalamus. However, this LEP is clearly active at LEPRs and challenges researchers to determine whether it has a role or not in avian physiology given its divergent expression in bird species.

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