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Leptin in teleostean fish, towards the origins of leptin physiology

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ABSTRACT

Teleostean leptin was first cloned in 2005, more than a decade after the discovery of mammalian leptin. The reason for this delay lies in the very poor primary sequence conservation (~13–25%) between mammalian and fish leptins. These low sequence conservations indicate a high degree of molecular evolvability and warrant a search for different and original functions of leptin in teleosts. Indeed, new and original insights are obtained because of the unique phylogenetic position of teleostean fish as the earliest vertebrates and because of their ectothermy, which means that teleosts are more flexible in changing their metabolism than mammals and leptin could play a role in this flexibility. Research during the last decade reveals that leptin is a truly pleiotropic hormone in fish and mammals alike, with functions among others in the regulation of food intake and body weight, development, but also in the regulation of the stress axis and acclimation processes to for instance low oxygen levels in the water. In this review, we provide an overview of the teleostean leptin work done in the last ten years, and demonstrate that the power of a comparative approach leads to new insights on the origins of leptin physiology.

Introduction

Early 2013 worldwide obesity had nearly doubled since 1980; over half a billion adults and 40 million pre-school children were obese. Shockingly, overweight and obesity nowadays kill more people worldwide than malnutrition does (WHO, 2013). This huge threat to human health has provided an impetus to the research on body weight regulation and energy homeostasis. Energy homeostasis requires an accurate match between energy intake, *i.e.* food intake and digestion, and energy expenditure, *i.e.* basal metabolic rate, physical activity and thermogenesis for endotherms. A key hormone coordinating this balance is the almost two decades ago discovered hormone leptin, named after the Greek root λεπτοζ meaning lean (Zhang et al., 1994). In mammals, leptin is produced by, and circulates in proportion to the amount of the white adipose tissue and acts in the hypothalamus on two primary types of neurons in the arcuate nucleus. One set of neurons is inhibited by leptin and expresses the orexigenic neuropeptide Y and agouti-related peptide (NPY/AgRP) (Broberger et al., 1998), whereas the other is stimulated by leptin and expresses the anorexigenic pro-opiomelanocortin (in fact the POMC-derived α-melanophore stimulating hormone, α-MSH) and cocaine and amphetamine regulated transcript (POMC/CART) (Elias et al., 1998). *Via* these two sets of neurons, and the secondary corticotropin-releasing factor (CRF) and thyrotropin releasing hormone (TRH) neurons in the paraventricular nucleus, leptin inhibits food intake and stimulates metabolism, *i.e.* energy expenditure, and by doing so restores energy balance under conditions of energy surplus (Morton et al., 2006; Schwartz et al., 2000). Besides this key role in energy metabolism, leptin has been shown to act as a pleiotropic hormone, with actions in the immune system (De Rosa et al., 2007), bone formation (Fu et al., 2006), angiogenesis (Anagnostoulis et al., 2008) and the stress response (Malendowicz et al., 2007; Roubos et al., 2012). This multitude and diversity of prime targets of leptin exemplify that understanding leptin's well-known epithet anorexigenic holds a grand challenge for physiologists.

The physiology of mammalian leptin with a focus on metabolism and food intake has been extensively reviewed (*e.g.* Keen-Rhinehart et al., 2013; Morton et al., 2006; Schneeberger et al., 2014). As the focus of our review lies on the recent advancements in the field of teleostean leptin physiology, we refer to these recent reviews for further reading on mammalian leptin physiology.

We strongly adhere to a comparative approach because of the diverse and versatile models provided by ectotherms, including teleosts, as they are less stringent in their metabolic homeostasis (Copeland et al., 2011; Denver et al., 2011; Londraville et al., 2014). Moreover, by studying teleostean fishes, the first true vertebrates on

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earth, one could gain insight in more 'original' functions of leptin, and indeed in recent years a rather different leptin physiology has emerged in teleostean fishes compared to mammals. We review the current insights in teleostean leptin physiology, and try to pinpoint future challenges in the field.

The discovery of teleostean *leptin* genes

After the cloning of the *leptin* gene in mammals, it took more than a decade to clone teleostean *leptin* orthologues (Huising et al., 2006a; Kurokawa et al., 2005). The reason of this delay lies in a very low amino acid sequence conservation; depending on the fish species between 13–25% homology compared with human leptin. Despite a dramatic difference in primary sequence, true orthology between fish and other vertebrate leptins was demonstrated based on conserved gene structure, stable clustering with other vertebrate *leptin* genes in phylogenetic analyses and conserved tertiary structure when modelled with the human leptin structure [PDB entry 1AX8 (Zhang et al., 1997)] as template (*e.g.* Gorissen et al., 2009; Huising et al., 2006a; Kurokawa et al., 2005).

A few years after the cloning of the carp *leptin* genes, another, highly divergent *leptin* gene was cloned in zebrafish and named *leptin-b*; as of then, the earlier discovered fish *leptins* are referred to as *leptin-a* (Gorissen et al., 2009).

Gene duplications, and whole (or larger parts of) genome duplications in particular, are considered to be the main force by which gene repertoires increase; because of a duplication event, one of the two gene copies can, on occasion, acquire a new function, whereas the other copy remains to fulfil the 'original' task. In general, if the two paralogues fail to differentiate their functions or their spatial or temporal expression patterns [in some cases gene dosage effects may result in the maintenance of both paralogues (Kondrashov et al., 2002)], one of the two paralogues will disappear as a result of redundancy. It is now well established that before the teleost-tetrapod split, two rounds of large scale (often referred to as whole) genome duplication (WGD) occurred (2R), followed by a teleost-specific genome duplication event (3R) (Meyer and Van de Peer, 2005; Sharman and Holland, 1996; Sidow, 1996), greatly increasing the gene repertoire of the early vertebrates. Many of the genes that originated in the third (*i.e.* teleostean) genome duplication evidently have disappeared in the course of evolution, as the estimated total gene number in teleostean genomes does not greatly exceed the number of genes in other vertebrate genomes (Aparicio et al., 2002). However, duplication events in teleosts are common, and some teleostean genes still exist in duplicate today, as in the class-I α-helical cytokine family (Huising et al., 2006b,c; 2005). Moreover, more recent genome duplications, or tetraploidisation events in common carp and salmonids result in up to four leptin paralogues in these species (Angotzi et al., 2013; Huising et al., 2006a; Rønnestad et al., 2010). One can argue, that these gene pairs must have differentiated in functionality or in temporal (ontogeny) and spatial (morphology) expression patterns, otherwise one of the two would have disappeared over time. However, also the duplicate *leptin* genes described within a fish species may be so extraordinarily different in primary amino acid sequence conservation (*e.g.* in zebrafish these genes share only a mere 24% amino acid identity) that it is hard to imagine that these *leptin* genes did not acquire different functionality and are only redundant. Indeed, the fact that they still exist and (one of the paralogues) have not gone lost is testament to their non-redundancy.

The dating of the '3R WGD' [∼300 Mya (Taylor et al., 2003; Volff, 2005)] at the very basis of teleostean evolution, means that probably all teleostean lineages have, or at least once had, duplicate *leptin* genes. Indeed, we were able to identify a *leptin-a* and *leptin-b* gene in medaka, a species whose evolutionary lineage separated ∼296 Mya (Hoegg and Meyer, 2005) from the cyprinid lineage of zebrafish and common carp, an observation that confirms this notion and anchors the *leptin* duplication very early in teleostean evolution (Gorissen et al., 2009). So, it is likely that duplicate *leptin* genes are a common feature among bony fishes (or in some cases were; we could retrieve only a single *leptin* gene in the Tetraodon genomes that might have lost one of the two *leptin* paralogues after their separation from the *Beloniformes* (medaka) lineage ∼186 Mya and their subsequent genomic reduction process). To generalize about the species-rich fish group is dangerous: analyses on striped bass (Won et al., 2012) and Chinese perch (He et al., 2013) yield only one *leptin* paralogue, while the orange spotted grouper possesses two *leptin* paralogues (Zhang et al., 2013), indicating that teleostean leptin phylogeny may be more complex than originally envisioned (Fig. 1). The coming elucidation of more genomes through modern, fast and affordable deep-sequencing techniques will shed light on the evolution of the leptin repertoire of teleosts. The estimated species number (∼35.000) provides a challenge and opportunity at the same time; fish are a rich source of evolutionary trials.

Leptin-a is found mainly in the liver (*e.g.* Gorissen et al., 2009; Huising et al., 2006a; Kurokawa et al., 2005; Rønnestad et al., 2010), whereas *leptin-b* has its highest expression in the ovaries and very much lower expression levels in the liver (Gorissen et al., 2009). Such a differential expression pattern is often testimony to differential functions. Indeed, upon a fasting challenge to zebrafish for up to one week, hepatic *leptin-b* but not *leptin-a* expression decreased; a result that suggests differential regulation and actions of the zebrafish leptin paralogues. Studies on mammalian models have firmly established that the single *Leptin* gene product in mammals is a truly pleiotropic cytokine which serves in the regulation of feed intake, feeding behaviour, metabolism, immunity, reproduction, bone metabolism and many more processes (Hausman et al., 2012; Matarese et al., 2010; Motyl and Rosen, 2012; Zaidi et al., 2012). The pleiotropy of course is co-determined by the as yet poorly understood receptor diversity and heterodimerization of cytokine receptor types (Liongue and Ward, 2007) as well as the highly complex and often promiscuous second messenger pathways associated (Gorissen et al., 2011).

Zebrafish leptin-b proteins are predicted to have a third cysteine residue that may or may not be available for intra- or intermolecular disulphide bridging [a feature also present in fish interleukin-11(a and b) proteins (Huising et al., 2005)]. This cysteine may facilitate some differentiation between the functions of zebrafish leptins. However, this extra cysteine is not a universal feature among teleosts, as other fish *leptin-b* sequences, including salmon (Rønnestad et al., 2010) and medaka (Kurokawa and Murashita, 2009), lack this cysteine, suggesting that if this amino acid is important in zebrafish leptin signalling, it may be species-specific. From our tertiary structure models we cannot conclude whether the third cysteine residue is at the very border of the protein surface and thus available for disulphide bridging or embedded in the protein interior (Gorissen et al., 2009).

Interestingly, we could find only one *leptin receptor* (*lepr*) gene in zebrafish, or indeed in any currently available teleostean genome we screened, including the very well annotated genomes of medaka, Tiger pufferfish and Green-spotted pufferfish (Gorissen et al., 2009), so the question remains if these leptin paralogues have different signalling capacities through a single type of leptin receptor. The vast difference in amino acid sequence between leptin-a and leptin-b, combined with the leptin receptor-binding properties (*i.e.* one binding site for leptin molecules), makes it difficult to envisage this mode of signalling. Indeed, when the binding energies of zebrafish and medaka leptin-a and leptin-b, bound to the leptin receptor of each species are calculated, the binding energy of leptin-a is considerably higher than that of leptin-b (Prokop et al., 2012).