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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).

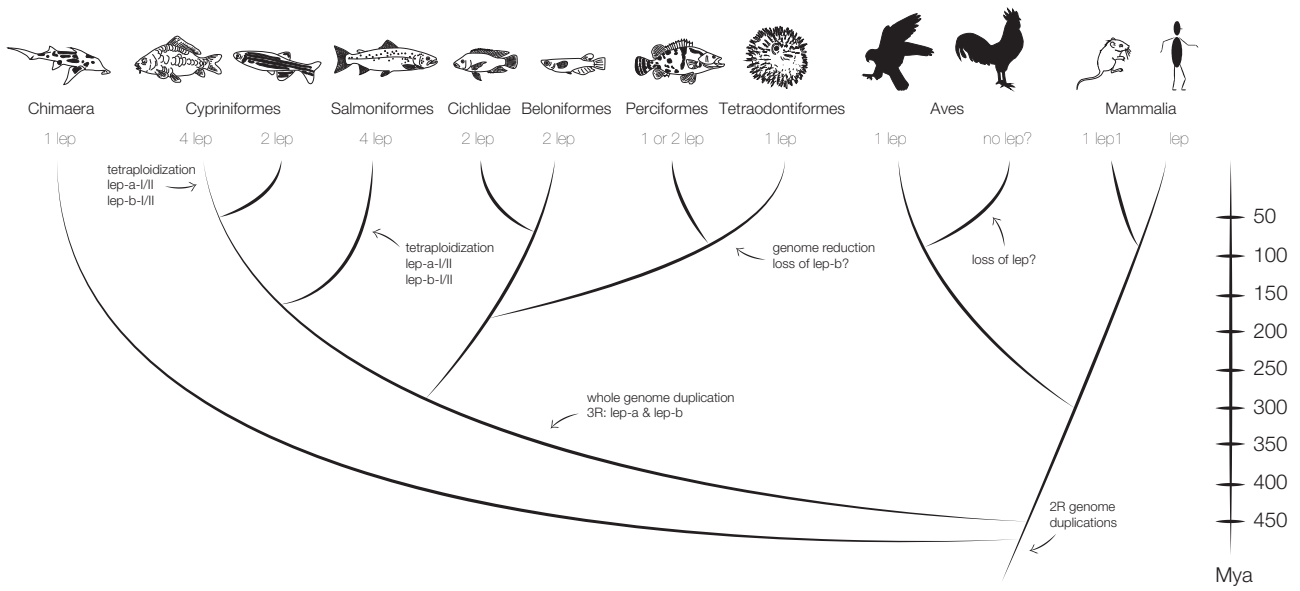

Fig. 1. Schematic phylogenetic tree of vertebrate evolution. Mammals and teleostean fish shared their last common ancestor ~450 million years ago (Mya). The finding of duplicate leptin paralogues in the medaka (*Beloniformes*) and zebrafish (*Cypriniformes*) dates the duplication event that gave rise to the *leptin-a* and *leptin-b* to ~296 Mya as these species shared their last common ancestor at that time point. The tetraploidization events of the carp and salmon genomes (~16 Mya and ~88 Mya respectively) are likely the events that, independently from one another, gave rise to paralogous *leptin-a-I* and *leptin-a-II* genes in carp and salmonids. Recently, the first *bona-fide* bird and shark *leptin* sequences were reported (Friedman-Einat et al., 2014; Prokop et al., 2014). Using these sequences, no *leptin* orthologues could be retrieved from the available bird genomes of chicken and turkey, supporting the hypothesis that *leptin* is missing in these species (Friedman-Einat et al., 2014). Divergence estimates are based on: (Hedges, 2002; Hoegg and Meyer, 2005; Macqueen and Johnston, 2014; Near et al., 2012; Volff, 2005; Zardoya and Doadrio, 1999).

Possibly, an as yet unknown receptor of the large family of type-I α-helical cytokine receptors acts downstream of one of the two leptin paralogues, or their actions are regulated on yet another level. Prokop et al. (2012) suggest that the multiple leptins found in fish may bind to the leptin receptor through altered hydrophobic interactions in a temperature related manner; an attractive hypothesis, since fish are ectotherms and thus their biochemistry is subjected to fluctuations in water temperature, and fluctuations in body temperature. Binding assays with leptin receptor and homologous recombinant leptin-a and leptin-b will elucidate whether both leptins act as ligand for the same leptin receptor, or that scenarios exist for leptin signalling in fish.

Multiple splice variants of the leptin receptor were identified in salmon (Rønnestad et al., 2010) and rainbow trout (Gong et al., 2013), but similar to the mammalian repertoire of leptin receptors, only one variant contains the full length sequence required for intracellular signal transduction, including ligand binding domain, transmembrane domain, Janus kinase (JAK) and signal transducer and activator of transcription (STAT) docking sites. In trout, fasting resulted in temporal and spatial changes in hepatic gene expression and plasma levels of the truncated leptin receptors (Gong et al., 2013). While no other data on the function of the truncated salmonid receptors are available at this moment, the fact that all five variants were identified in the same tissue (pituitary gland) in Atlantic salmon (Rønnestad et al., 2010) suggests a rather complex leptin system, at least in the pituitary gland. Indeed, some of the splice variants could function as a soluble leptin binding protein (Gong et al., 2013), thereby modulating the endocrine and/or paracrine output of leptin from the liver and/or pituitary gland.

Physiology

Food intake and body weight

Early experiments with common carp indicated a fundamentally different leptin physiology compared to mammals. Although hepatic *leptin* (*-a*) expression showed a marked post-prandial increase in expression similar to that observed in mice (Saladin et al., 1995), *leptin-a* mRNA levels did not change upon long-term fasting for up to six weeks (Huising et al., 2006a). This links the liver to the regulation of short-term food intake in teleostean fish. Together with the gut, the liver is a logical place to integrate information regarding food intake, as it directly receives blood from the gut *via* the portal vessel system, and the increase in *leptin-a* follows the post-prandial increase in glucose and drop in non-esterified fatty acids (NEFA) (Huising et al., 2006a). If the increase in *leptin-a* expression is regulated directly *via* nutrients, or *via* other (humoral) regulators such as insulin or cholecystokinin (CCK) remains unanswered.

Since the first experiments regarding leptin and food intake in carp, many more studies have explored the function of leptin with respect to food intake. Interestingly, some studies provide clear evidence for increased leptin (protein) levels after a period of fasting (Fuentes et al., 2012; Kling et al., 2009). This would contradict a role for leptin as an adipostat, and suggests an orexigenic, not an anorexigenic mode of action. However, intraperitoneal (ip) and intracerebroventricular (icv) injections of both heterologous and homologous leptin proteins in fish consistently decrease food intake (de Pedro et al., 2006; Li et al., 2010; Londraville and Duvall, 2002; Murashita et al., 2011, 2008; Volkoff et al., 2003). Moreover, the increase in plasma leptin levels seen in rainbow trout and salmon during fasting was obtained using the same radioimmunoassay based on a salmonid leptin fragment of 14 amino acids (Kling et al., 2009). A(t least a) second leptin assay for salmon is needed in order to validate these results and be able to firmly conclude a rise in leptin plasma levels as a result of fasting. Recently, a homologous ELISA for tilapia leptin-a was reported (Douros et al., 2014) but no plasma levels have been compared between fed and fasted tilapia yet. Indeed, more homologous assays are needed within and among fish species, to gain a better understanding of the meaning of circulating leptin levels under different conditions of energy status.

Recently, interesting new tools to study leptin physiology in fishes were reported: a knock-down *lep-a* zebrafish (Liu et al.,

2012) and a medaka leptin receptor mutant (Chisada et al., 2013). One could regard the latter model as a teleostean equivalent of the famous *db/db* mouse model. In the hypothalamus of this *lepr*$^{-/-}$ mutant orexigenic signals *npy* and *agrp* were constantly up-regulated, whereas *pomc1* expression was down-regulated. Mutant medaka were characterised by higher feed intake in both post-juveniles and adult fish. Interestingly, this increase in food intake corresponded with a higher growth rate at the post-juvenile stage, but did not change adult body size (Chisada et al., 2013). Perhaps even more striking is that this mutant medaka is characterised by increased visceral fat deposition, whereas fat content of liver and muscle did not change. Visceral fat does not express high amounts of *leptin* in most fish, and even in mammals, the contribution of visceral fat to the plasma leptin tonus is limited (Woods et al., 2003). Perhaps the storage in the form of visceral fat is some kind of escape route to store the excess energy. How this amount of energy then contributes to (and signals to the brain regarding to) energy reserves in the periphery remains unclear. Given that insulin levels are better correlated to visceral fat than leptin levels are (Woods et al., 2003), the contribution of insulin to the long-term regulation of food intake in fish and its synergy with leptin provide an interesting avenue for future research. Also, because fish lack the layer of isolating fat tissue, fish models could provide insight in the differences between visceral fat and subcutaneous (isolation) fat and their respective contributions to obesitas related pathology.

Leptin and metabolism

Leptin plays a major role in the regulation of metabolism in fish (Dalman et al., 2013). Since fish are ectotherms, a drop in water temperature or in oxygen availability – events that happen regularly and easily in the life of a fish – induces a concomitant drop in their internal metabolism, so-called metabolic suppression (vanRaaij et al., 1996; Zhou et al., 2001). Besides migration (if possible) to water with a higher oxygen saturation or lower temperature in order to conserve energy and reduce oxygen consumption, inhibition of feeding (Bernier and Craig, 2005; Bernier et al., 2012; Boutilier et al., 1988; Buentello et al., 2000; Chabot and Dutil, 1999; Pedersen, 1987; Pichavant et al., 2001; Ripley and Foran, 2007; Zhou et al., 2001) is one of the main responses of fish exposed to hypoxic water conditions, and explains at least partially the growth impairment seen under such conditions (Chu et al., 2010). Leptin secretion by mammalian adipocytes increases under hypoxic conditions and involves mediation by the oxygen-sensitive transcription factor hypoxia-inducible factor-1α (HIF-1α) (Bartella et al., 2008; Snyder et al., 2008; Wang et al., 2008). A similar HIF-1α pathway has been found in fish (Kajimura et al., 2006; Nikinmaa and Rees, 2005), which prompted Chu et al. (2010) to study gene expression of *leptin-a* in zebrafish . Indeed, hypoxia increased *leptin-a* mRNA expression in the liver of adult zebrafish. Moreover, overexpression of *hif-1α* mRNA following exposure to cobalt in developing zebrafish embryos markedly increased *leptin-a* expression (Yu et al., 2012), and this is taken as evidence that HIF-1α is involved in the regulation of *leptin-a* . In addition to an increase in *leptin* mRNA, the adaptation to hypoxia results in a dramatically altered haematology (*i.e.* increased haematocrit and haemoglobin content to enhance oxygen carrying capacity) and changes in expression levels of signals of the hypothalamic feeding circuitry *npy*/*agrp* and *pomc*/*cart* to reduce food intake, lower metabolism, and save energy to relocate towards processes needed to cope with the change in environmental conditions (Bernier et al., 2012). Besides being involved in the adaptation processes to environmental hypoxia, a recent study (Macdonald et al., 2014) demonstrates that leptin is involved in the anorexic response to disease-mediated hypoxia. Trout infected with the haemoflagellate *Cryptobia salmositica* show a marked decrease in appetite, which is (at least in part) regulated by an increase in hepatic *leptin-a1* expression and a shift from orexigenic (*npy* and *agrp*) towards anorexigenic (*pomc-a2*) gene expression in the hypothalamus.

An important observation from Bernier et al. (2012) and MacDonald et al. (2014) is that the primary cue for the increase in *leptin-a* expression is hypoxia and not the associated reduction in feed intake (evidenced by proper pair-feeding controls). As discussed by Huising et al. (2006a), without a need to thermo-regulate, fish have large scope (*e.g.* by behavioural responses, seeking appropriate niches with different temperature or oxygen level) to adjust/lower their metabolic rate for prolonged periods when confronted with food shortage. Consequently, there is a less rigid need to signal a deficit in nutrient availability. However, the survival in conditions of low temperature and low oxygen content of the water, which often correlates directly to energy availability, does require a precise match between energy utilisation and intake, a balance that might be regulated by leptin. Understanding fish in this respect may hold crucial information for anaesthesiologists challenged with conditions of temperature and metabolic instability during (lasting) surgery. The well-studied crucian carp (*Carassius carassius*), arguably the most hypoxia-tolerant vertebrate known, should be studied for the role of leptin in its physiology (Liao et al., 2013) in this context.

Leptin and reproduction

Early studies that made use of high doses of recombinant human leptin (10^{-8} M – 10^{-6} M) suggested a role for leptin in the regulation of reproduction: administration of high concentrations of recombinant human leptin in European sea bass and rainbow trout (Peyon et al., 2001; Weil et al., 2003) shows that leptin might increase LH and FSH secretion from pituitary gland cells *in vitro*. Recently, with more sophisticated tools available, Trombley and Schmitz (2013) show that hepatic *leptin-a1* expression increases in mature, male, Atlantic salmon parr (Trombley and Schmitz, 2013). However, the same study reported no differences in plasma leptin levels, which suggests that the other *leptin* paralogues contribute substantially to plasma leptin levels. A more recent study (Trombley et al., 2014) shows upregulation of both *leptin-a* paralogues in salmon, but lacks expression levels of *leptin-b* genes or (total leptin) plasma levels. Although no qPCR-data on *leptin-b* tissue expression profiles are available for salmon, the high expression of *leptin-b* in zebrafish ovaries (Gorissen et al., 2009) argues for a role of leptin-b in the regulation of female reproduction. It is clear that only the tip of the iceberg of the role of leptin in sexual maturation of fish has been revealed, but this promises to be a promising future research area.

Leptin and the stress-axis

Direct links between leptin and the stress-axis have been established in mammals (Roubos et al., 2012). In fish, the stress-axis 'starts' in the hypothalamic pre-optic area (POA), where CRF is co-produced with CRF-binding protein (CRF-BP) and released by axons in the neighbourhood of corticotrope cells in the *pars distalis*. These corticotrope cells produce the pro-hormone pro-opiomelanocortin (POMC) as a response to stimulation by CRF, which is processed in the *pars distalis* into adrenocorticotropic hormone (ACTH). POMC in the *pars intermedia* will be processed further into α-MSH – which plays a poorly understood function in coping with chronic stress. ACTH travels *via* the blood to the interrenal cells in the head kidney (the teleostean equivalent of the mammalian adrenal cortex) where cortisol is produced in response to ACTH stimulation. In teleostean fish, which do not produce aldosterone

as mineralocorticoid, cortisol serves in two major processes: regulation of hydromineral balance and the redistribution of energy flow towards adaptation processes to cope with a certain stressor. The former process is conveyed through the mineralocorticoid receptor (MR), whereas the latter is delivered through the glucocorticoid receptor (GR) (Wendelaar Bonga, 1997).

Recombinant human leptin attenuates the stress axis of common carp at multiple levels (Gorissen et al., 2012). At the level of the head kidney, the absolute amount of cortisol released is lowered under elevated leptin tonus, whereas the relative stimulation by ACTH is not affected. We observed high *leptin-a* and *leptin-b* levels (Gorissen et al., 2009) and a high expression of *leptin receptor* (Rønnestad et al., 2010), and our unpublished results) in the pituitary gland, providing support for a role of leptin in pituitary gland hormone output. In an *in-vitro* perifusion set-up, we observed a rapid and prolonged decrease of ACTH release by administration of recombinant human leptin, both constitutive and CRF-induced and regulated release. Together with observations from Douros et al. (2014) and Tipsmark et al. (2008) that leptin stimulates prolactin and prolactin inhibits leptin, this suggests that leptin could be a major regulator of pituitary gland output, both of the stress-axis and the osmoregulatory axis (prolactin is essential for fish survival in fresh water). Indeed, our unpublished data (Gorissen, van den Akker, Zethof, Flik, McCormick, Ebbesson and Nilsen, unpublished observations) and a study by Baltzegar et al. (2014) confirm a regulatory role for leptin in osmoregulation of salmon and tilapia respectively.

As discussed earlier (see Section Leptin and metabolism), *leptin* expression increases during hypoxic conditions. In these conditions where energy expenditure is decreased dramatically, launching a large stress response could be counterproductive, as cortisol and metabolism are intimately linked. Leptin may convey information on energy status and serve to downplay the stress response in these conditions, and contribute to the coordination of the delicate balance between eustress and distress.

Perspectives

Already, after a mere ten years of research, the teleostean models provide us with unique virtues and a huge versatility and variability to shed new light on the origins, and original functions, of leptin, a pleiotropic hormone with a key and central role in the acclimation to environmental challenges that require energy reallocation. Leptin does play substantially different roles in the physiology of fish than the role of leptin that we associate with actions in mammals.

Although a lot of work has been done, several important questions have yet to be addressed: *e.g.* what are the interactions between leptin and other 'metabolic' hormones like insulin, ghrelin and CCK? Does the single leptin receptor serve as receptor for both leptin-a and leptin-b, are mechanisms downstream of the receptor determining factors or maybe is there an as yet undiscovered leptin receptor in the ample genome repertoire of fish? Moreover, interesting avenues of research not further discussed here include leptin's effect on angiogenesis (that may be important in the adaptive response to hypoxia), leptin's involvement in the immune system (Mariano et al., 2013) and bone physiology.

Now almost a decade after the discovery of leptin in teleostean fishes, all components of the mammalian leptin system have been identified in fish: multiple leptin genes, a single leptin receptor (with different splice variants and accompanying different signalling capacities) and a leptin receptor-overlapping transcript (LEPROT) (Kurokawa et al., 2008). Also, mutant models are generated and used to study the effects of leptin or its receptor in fish. With this toolkit, comparative leptin research can elucidate further the (pleiotropic) role of leptin in the earliest vertebrates, and with that, shed a new and original light on the origin and evolution of the system that plays such an important part in vertebrate energy homeostasis. A take-home-message from our studies is the notion that we should start thinking about vertebrate physiology with fish as models where vertebrate endocrine repertoires are often original and best represented.

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