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Recombinant human leptin attenuates stress axis activity in common carp (*Cyprinus carpio* L.)

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ABSTRACT

Proper functioning of the endocrine stress axis requires communication between the stress axis and other regulatory mechanisms. We here describe an intimate interplay between the stress axis and recombinant human leptin (rhLeptin) in a teleostean fish, the common carp *Cyprinus carpio*. Restraint stress (by netting up to 96 h) increased plasma cortisol but did not affect hepatic leptin expression. Perifusion of pituitary glands or head kidneys with rhLeptin revealed direct effects of rhLeptin on both tissues. RhLeptin suppresses basal and CRF-induced ACTH-secretion in a rapid and concentration-dependent manner. The rhLeptin effect persisted for over an hour after administration had been terminated. RhLeptin decreases basal interrenal cortisol secretion *in vitro*, and by doing so attenuates ACTH-stimulated cortisol production; rhLeptin does not affect interrenal ACTH-sensitivity. Our findings show that the endocrine stress axis activity and leptin are inseparably linked in a teleostean fish, a notion relevant to further our insights in the evolution of leptin physiology in vertebrates.

1. Introduction

Since the initial cloning of the *Obese* (*Ob*) gene in mice [52], its key role in the regulation of food intake and body weight has been well established [36,42], fuelled by the increasing occurrence of obesity throughout the (Western) world. In mammals, leptin is primarily produced and secreted by white adipose tissue [52] and circulates in proportion to the amount of body fat. From the blood stream, leptin is shuttled across the blood brain barrier coupled to a truncated soluble form of the leptin receptor [24] and signals to the arcuate nucleus (ARC). The peripheral signals regarding energy status are integrated and transmitted by arcuate neurons to higher order brain centres (among others the paraventricular nucleus, PVN) to terminate food intake, increase metabolism [10,36] and by doing so, guarantee energy homoeostasis. Besides its key role in the regulation of food intake and body weight, in recent years leptin was shown to be a truly pleiotropic hormone, and has been implicated to serve in *e.g.*, the immune response [9,31], bone formation [15] and the stress response [30,40].

Early studies on leptin in fish revealed the liver as a main site of expression; further it was also shown that *leptin* expression does not change after fasting or feeding to satiation for up to six weeks [22]. Since this first paper on the physiology of a teleostean leptin, a rather different leptin physiology in fish compared to mammals has emerged from studies on zebrafish [18], Atlantic salmon [38], rainbow trout [26] and Arctic char [12]: in a nutshell, these studies support the notion of the liver rather than adipocytes as the primary site of leptin production; these studies further suggest that the role of leptin in energy homoeostasis in fish may differ from the accepted role in mammals.

The stress response in fish shows many similarities to that of terrestrial vertebrates [50]. Primary stress hormones include the catecholamines and the signals of the hypothalamic - pituitary – interrenal (HPI) axis. In response to a stressor, corticotropin-releasing factor (CRF) produced in the nucleus preopticus is sent via axons to corticotrope cells in the pituitary pars distalis where it initiates the release of adrenocorticotropic hormone (ACTH). ACTH is transported to the interrenal cells in the head kidney and triggers production and release of cortisol, the main end product of the stress axis [11,50]. In fish, cortisol exerts a plethora of functions. Key targets for cortisol are the gills, liver, intestine and kidneys. These organs reflect the two major actions of cortisol in fish: regulation of (1) hydromineral balance and (2) energy partitioning. Fish do not produce aldosterone, they lack aldosterone synthase; dependent on the types of receptor (mineralocorticoid receptor [MR] or glucocorticoid receptor [GR]) present in a cell, cortisol exerts mineralo- and/or glucocorticoid actions [32,44,50].

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Table 1
Nucleotide sequences of the primers used for RT-qPCRs.

Gene	Accession#	FW 5′ → 3′	RV 5′ → 3′
Leptin-a-I	AJ868357	CATATTGATTTGTCCACCCTTCTG	CCATTAGCTGGCTCCTTGGAT
Leptin-a-II	AJ868356	AGATACGCAACGATTTGTTCACA	GCGTTGTTCTCCAAGAAAGCA
Elongation factor 1α	AF485331	CACGTCGACTCCGGAAAGTC	CGATTCCACCGCATTTGTAGA
40S	AB012087	CCGTGGGTGACATCGTTACA	TCAGGACATTGAACCTCACTGTCT

Stressed fish reduce their food intake and this is considered to be the main reason for impaired growth of stressed fish [3]. Cortisol released during distress is assumed to be the primary mediator of impaired growth [37]; cortisol influences growth of fish in several ways, *e.g.*, by diversion of energy away from anabolic processes [8,47] or by the reduction of intestinal nutrient absorption [35].

The energy partitioning actions of stress axis hormones, and a possible role for leptin in fish energy metabolism makes one predict cross-talk or bidirectional communication between leptin and the stress axis. In mammals, numerous studies describe effects of leptin at multiple levels of the HPA-axis: *e.g.*, leptin decreases expression of CRF in the paraventricular nucleus and reverses the CRF-stimulatory effect of adrenalectomy in *ob/ob* mice [1,21], augments plasma ACTH concentrations in response to electrical shocks in rats [25], decreases adrenal steroidogenesis in rat adrenocortical cell lines [20,49] and lowers plasma corticosterone levels in mice [21]. To date, no effects of leptin on the stress axis of any non-mammalian species have been reported.

The demonstration of *leptin* genes in teleosts provides us with opportunities to study the physiology of this hormone in the earliest vertebrates, and gives us new and original insights in leptin physiology. We here demonstrate for common carp, *Cyprinus carpio* interactions between the stress axis and rhLeptin. Netting stress for up to 96 h does not influence hepatic leptin-a[1] mRNA levels, recombinant human leptin inhibits both basal and CRF-induced ACTH-secretion and decreases interrenal basal cortisol secretory activity, and these observations substantiate a direct effect of rhLeptin on stress axis components. Taken together, these findings show that the stress axis and leptin are intimately related in common carp.

2. Materials and methods

2.1. Animals

Common carp (*C. carpio* L.) was obtained from a commercial farm in Valkenswaard, The Netherlands. Fish were kept in glass tanks containing 150 L recirculated (80%; 20% fresh input of tap water), biofiltered and UV-treated Nijmegen tap water at 23 °C. Fish were fed 2% of the estimated body weight daily with commercial fish food (Trouvit). Experimental design obeyed Dutch legislation and was approved by the ethics committee of the Radboud University Nijmegen.

2.2. Restraint stress

To examine *leptin* expression during stress, we applied net restraint as stressor. Carp ($n = 8$ per tank; both male and female fish were used) were housed in identical 150 L tanks. Fish were fed 2% of the estimated body weight until restraint was given. On $t = 0$ "control fish" were sampled and "restraint fish" were confined in a net. Restrained fish and pair-fed controls did not receive food from $t = 0$ until the end of the experiment. At defined time points, fish were sampled as described below.

2.3. Sampling

Fish were anaesthetised in 0.1% (v/v) 2-phenoxyethanol (Sigma, St. Louis, USA) and blood was collected by puncture of the caudal veins with a tuberculin syringe fitted with a 25-G needle; heparin was used as anticoagulant. After blood collection, the fish were killed by cutting the spinal cord caudal of the opercula. For perifusion, left and right head kidneys were collected, as was the pituitary gland, which was bisected into pars distalis and pars intermedia. For quantification of mRNA levels, collected tissues were flash-frozen on dry ice and kept at −80 °C until processing.

2.4. Determination of plasma glucose and NEFA levels

Plasma glucose and non-esterified fatty acids (NEFA) were determined by use of the "Glucose liquicolor" and "NEFA-C" method, respectively, according to the manufacturer's instructions (Wako chemicals, Neuss, Germany).

2.5. Perifusion

Perifusion (also called superfusion) experiments were performed as previously described [34]. Briefly, head kidney or the pituitary pars distalis were placed in a perifusion chamber and perifused with carbogen-saturated (95% O_2/5% CO_2) 15 mM HEPES/Tris-buffer (pH 7.4) containing 128 mM NaCl, 2 mM KCl, 2 mM $CaCl_2$, 0.25% (w/v) glucose, 0.03% (w/v) bovine serum albumin and 0.1 mM ascorbic acid. Peptides and amines of interest – rhLeptin, human ACTH[1–39], ovine CRF and dopamine – were all obtained from Sigma–Aldrich (St. Louis, USA) and were dissolved in the perifusion medium to the desired concentration. Medium was perifused at 30 μl/min. Perifused medium was collected over 5- or 15-min intervals and then immediately placed on ice until further analysis by radioimmunoassay (RIA).

2.6. Determination of plasma hormone concentrations

ACTH was quantified by RIA as previously described [34]. In short, the incubation mixture consisted of 25 μl samples (in duplicate) or standards (in triplicate) with 100 μl of 1:1000 diluted antibody (raised against human ACTH[1–24]; Biogenesis, Poole, UK). After 24 h at 4 °C, 100 μl of ^{125}I-labelled human ACTH[1–39] was added and incubated for another 24 h at 4 °C. Bound antigens were incubated for 30 min at room temperature with 100 μl of 1:20 diluted goat anti-rabbit IgG (Biotrend, Köln, Germany) in 0.005% (w/v) rabbit IgG (Sigma). To precipitate immune complexes, one ml ice-cold precipitation buffer (7.5% (w/v) polyethylene glycol 6000) was added and centrifuged for 10 min at 2000*g* and 4 °C. The supernatant was aspirated and pellets were analysed for gamma-emission.

Cortisol was measured using a RIA in a 96-well plate. All wells except the 'non-specifics' received 100 μl cortisol antibody

[1] Zebrafish expresses two leptin genes, *leptin-a* and *leptin-b* (Gorissen et al., 2009); common carp, a tetraploid species, expresses two copies of *leptin-a* and *leptin-b*. The expression of leptin-b in carp liver is essentially undetectable under all conditions tested so far (unpublished); for that reason we address only *leptin-a (I&II)* in this study.

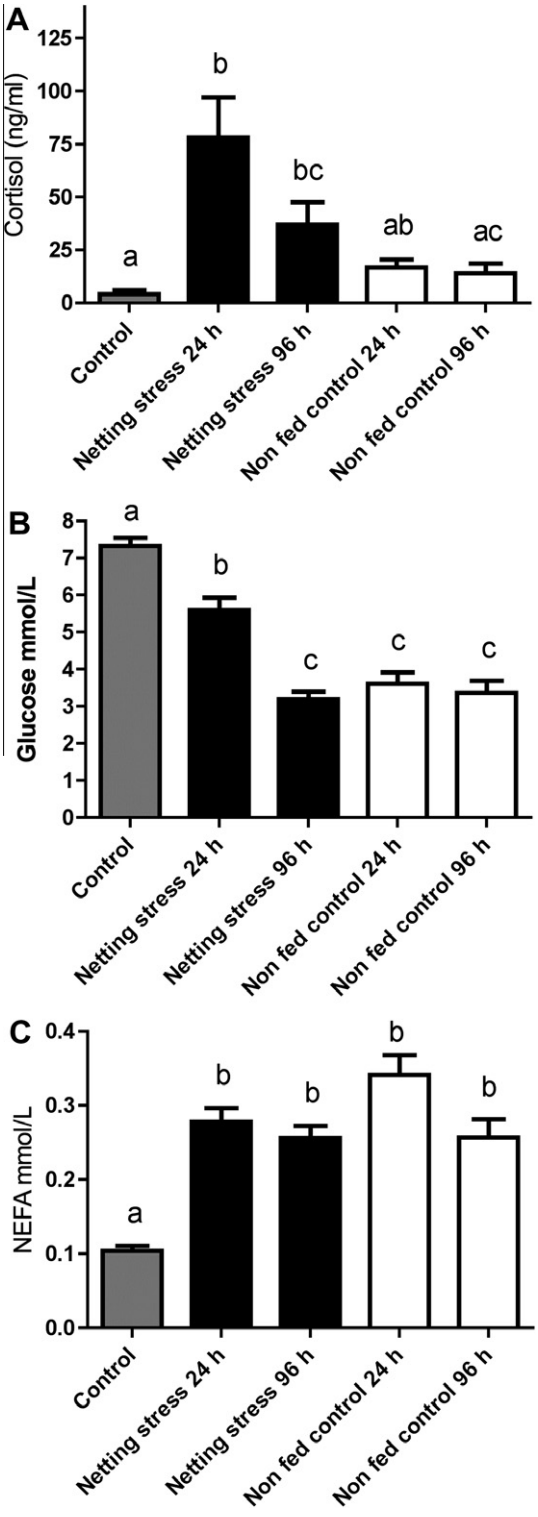

Fig. 1. Effect of netting restraint (black columns) for 24 and 96 h on plasma cortisol (A), glucose (B) and NEFA (C) levels compared to control (grey column) and non-fed controls (white columns). Values represent means ± S.E. ($n = 10$). Columns that do not share a common letter differ significantly from one another. $P < 0.05$ was accepted as fiducial limit.

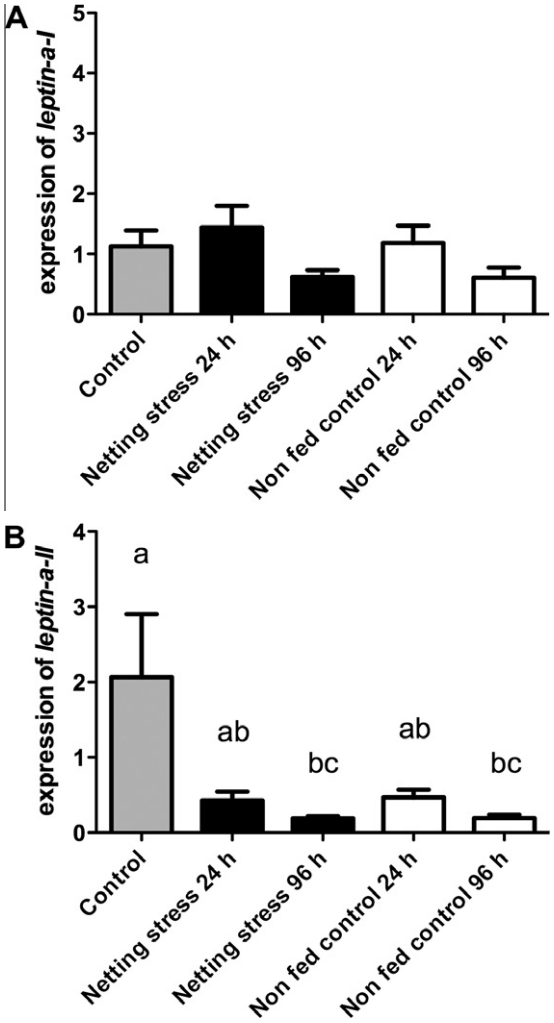

Fig. 2. Effect of restraint (black columns) for 24 and 96 h on hepatic *leptin-a-I* (A) and *leptin-a-II* (B) expression. Gene expression presented here was first normalised to the expression of *elongation factor 1α* and then presented relative to controls. Values represent means ± S.E. ($n = 10$). Columns that do not share a common letter differ significantly from one another ($P < 0.05$).

(Cortisol Antibody[xm210] monoclonal and IgG purified (Abcam); 1:2000 in 50 mM $NaHCO_3$, 50 mM NaH_2CO_3, 0.02% NaN_3, pH 9.6) and were incubated overnight at 4 °C. The following day, the plates were washed three times with 200 µl/well wash buffer (100 mM Tris, 0.9% NaCl, 0.02% NaN_3). Subsequently, non-specific sites were blocked by the addition of 100 µl blocking buffer (100 mM Tris, 0.9% NaCl, 0.02% NaN_3, 0.25% Normal Calf Serum) to each well. Plates were covered and incubated for one h at 37 °C. Subsequently, 10 µl of standard (4–2048 pg cortisol/10 µl assay buffer containing 100 mM Tris, 0.9% NaCl, 0.1% 8-anilino-1-naphthalenesulfonic acid, 0.02% NaN_3) or 10 µl of undiluted plasma or perifusion medium was added to designated wells. Non-specifics and B_0 received 10 µl assay buffer. After the addition of standards and samples, 90 µl (333 Bq) of ^{3}H-hydrocortisone (PerkinElmer, USA, 1:10,000 in assay buffer) solution was added to all wells. Plates were incubated overnight at 4 °C. The plates were then washed three times with wash buffer. After the final wash step, all wells received 200 µl of 'Optiphase hisafe-3 scintillation liquid' (PerkinElmer, USA) and were covered. Beta-emission was quantified by a 3 min count per well using a Microbeta Plus (Wallac/PerkinElmer, USA).

2.7. Gene expression analysis

Total RNA was isolated with Trizol (Invitrogen, Carlsbad, USA) according to the manufacturer's instructions and diluted as required in ultrapure water. RNA concentration and purity was measured by nanodrop spectrophotometry. Samples were treated with

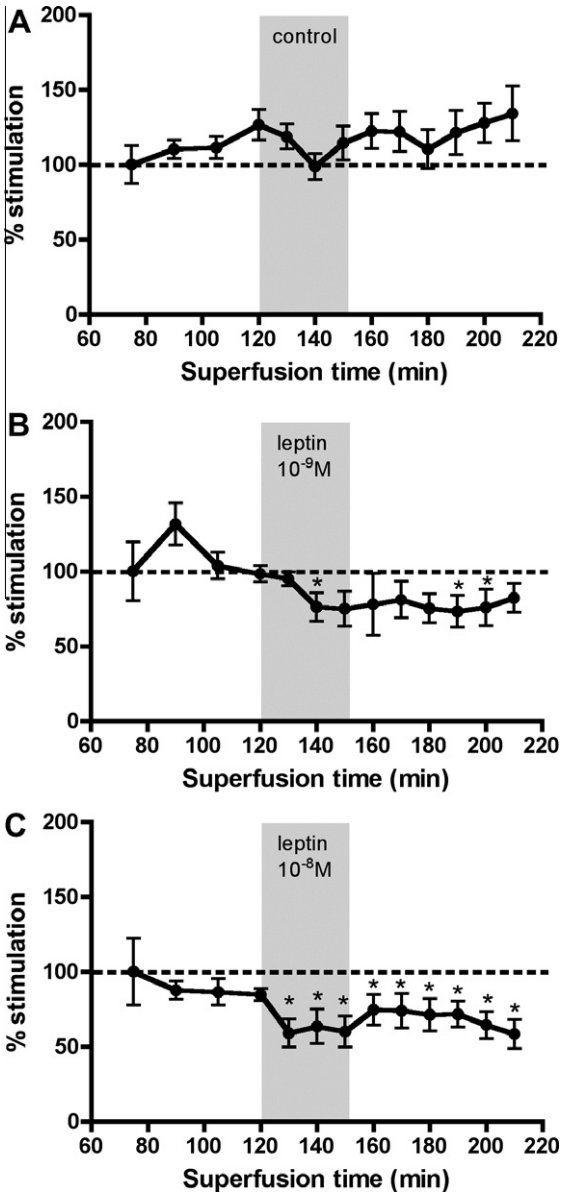

Fig. 3. In-vitro release of ACTH from anterior pituitary glands in the absence (A, controls $n = 15$), and presence of 10^{-9} M leptin (B, $n = 8$) and 10^{-8} M leptin (C, $n = 6$). Values represent the mean percentage ACTH release relative to basal, unstimulated release ± S.E. Asterisks indicate significant difference from basal release ($^{*}P < 0.05$).

DNase (Invitrogen, Carlsbad, USA) to remove genomic DNA. One µl of total RNA was added to 1 µl of DNase I and incubated in a total volume of 10 µl at room temperature. DNase was then inactivated by addition of 1 µl EDTA (25 mM) and incubation for 10 min at 65 °C to simultaneously linearise the RNA. To each sample 300 ng random primers, 4 µl 5× First Strand buffer, 1 µl 10 mM dNTP mix, 2 µl 0.1 M DTT, 1 µl RNaseOUT and 200 U of Superscript II Reverse Transcriptase (all from Invitrogen) was added and incubated for 50 min at 42 °C to synthesise first strand cDNA. cDNA was kept at -20 °C. Relative gene expression was assessed by real-time qPCR. In short, 5 µl of 5× diluted cDNA was used as template in a reaction with 12.5 µl SYBR Green Mastermix (Applied Biosystems), 5 µl forward primer, 5 µl reverse primer (Table 1) and 1.5 µl dH_2O. qPCR (10 min 95 °C, 40 cycles of 15 s 95 °C and 1 min 60 °C) was carried out on a 7500 Real Time PCR system (Applied Biosystems). Data were analysed with the 'ΔΔCt-method'. Dual internal standards (40-S and elongation factor 1α (ELF1α)) were used in all PCRs and results were confirmed to be similar following standardization to either gene. Results standardised for ELF1α are shown.

2.8. Statistics

Statistical analyses were performed with Graphpad Prism 4.0 (Graphpad Software Inc., La Jolla, USA). Data were tested for normality with the D'Agostino & Pearson omnibus normality test. Differences were evaluated with one-way ANOVA, followed by Tukey's test to determine which means differed significantly from each other. The Kruskal–Wallis test was applied in the case of non-Gaussian distribution of data, or an inequality of variances, followed by a Dunn's post test. $P < 0.05$ was accepted as fiducial limit.

3. Results

3.1. Netting stress for 24 and 96 h increases plasma cortisol

Netting for 24 or 96 h increased plasma cortisol levels compared to control levels in fed fish (Fig. 1A). However, during netting fish are not fed; to discriminate between the effects of netting and the effects of fasting, we included pair-fed (*i.c.* fasted) control groups in our experiment. Cortisol levels in the fasted groups were slightly but not significantly elevated compared to levels in fed controls.

Glucose and NEFA were assessed to gain more insight in the metabolic status. Fasting for 24 or 96 h decreased plasma glucose levels (from approximately 7–4 mM); (Fig. 1B). In general, the decreased glucose levels and concomitantly increased NEFA levels (Fig. 1C) indicate a shift from carbohydrate metabolism to fat metabolism, a characteristic of carp challenged with fasting.

3.2. Hepatic leptin-a-I/II expression after netting stress

Netting nor fasting for up to 96 h up- or down-regulated hepatic *leptin-a-I* (Fig. 2A). However, *leptin-a-II* expression had decreased after 96 h of food deprivation (Fig. 2B). This down-regulation of *leptin-a-II* is both observed in stressed fish and non-fed controls and this indicates that this is a fasting effect, rather than a reaction to the stressor.

3.3. RhLeptin attenuates basal and CRF-induced ACTH release

Fig. 3A confirms an earlier report [34] where it was shown that an ectopic pituitary pars distalis secretes ACTH spontaneously; this secretion decreases concentration-dependently by addition of recombinant human leptin (10^{-9}–10^{-8}; Fig. 3B and C). The effect of rhLeptin addition on ACTH secretion is rapid (effect seen within 10 min) and persists for at least an hour, as ACTH secretion remains suppressed for at least 1 h after removal of the rhLeptin tonus.

We tested two CRF concentrations (10^{-7} and 10^{-6} M) on the ACTH release. Whereas CRF administered at a 10^{-7} M modestly increased ACTH secretion (Fig. 4A), 10^{-6} M CRF markedly increased ACTH release (Fig. 4B). RhLeptin (10^{-8} M) administration completely abolished CRF-induced ACTH secretion (Fig. 4C–D).

3.4. RhLeptin decreases basal cortisol secretion, but does not affect ACTH-sensitivity

We exposed head kidneys to ACTH (5×10^{-8} M). From each fish, either the left or the right head kidney was exposed to 10^{-7} M rhLeptin from the start of the experiment whereas the other head kidney (from the same fish) served as control. Administration of rhLeptin from the beginning of the experiment decreased basal

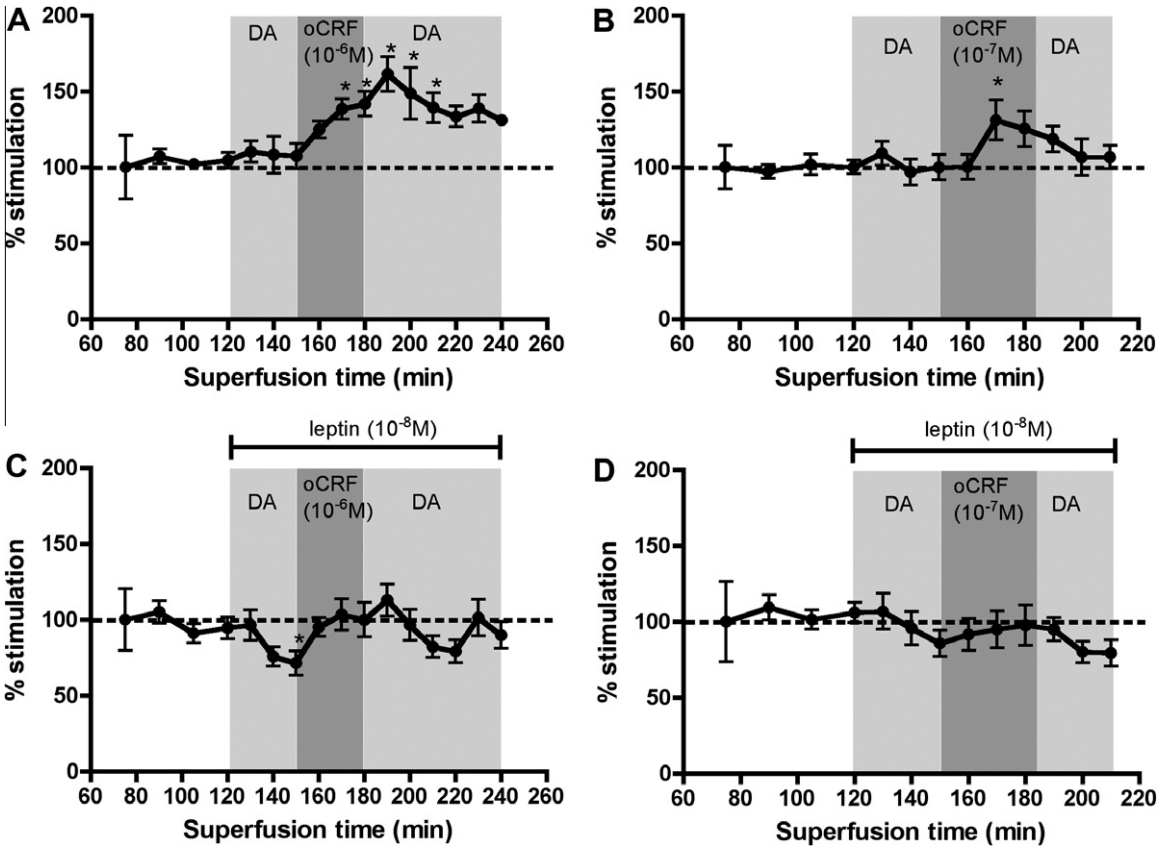

Fig. 4. In-vitro release of ACTH from anterior pituitary glands. We assessed ACTH release induced by two concentrations of CRF, 10^{-6} M (A) and 10^{-7} M (B), and the effect of administration of leptin, 10^{-8} M (C and D), on regulated ACTH release ($n = 8$). Values represent the percentage ACTH release relative to basal, unstimulated release ± S.E. Asterisks indicate significant differences with basal release. Differences were considered to be significant when $P < 0.05$.

cortisol release (Fig. 5B), but not the percentual increase in cortisol release upon ACTH stimulation (*i.e.* the relative cortisol secretion calculated by manually adjusting the cortisol secretion just before the ACTH stimulation to 100%; Fig. 5A).

4. Discussion

The endocrine stress axis in fish is activated during both eustress and distress [50]; interaction of the stress axis with other regulatory mechanisms targeting common metabolic pathways is pivotal for proper functioning of the stress axis during periods of fasting and (over-)feeding, illness and during reproduction. In the past regulatory axes were often seen as independent and discrete entities; nowadays it is widely recognised that regulatory systems interact with each other and even share ligands and receptors to allow for bi-directional communication [5]. Important communication occurs between the stress axis of fish and the thyroid axis [16,17], the reproductive axis [41] as well as the immune system [33] and this is not different from the situation in higher vertebrates including mammals.

Cortisol is a recruiter of carbohydrate fuels, and replenishes glycogen stocks [35]. Although for fish the role of leptin is not as firmly established as in mammals, it plays a key role in energy metabolism and is therefore likely to interact with the stress axis. In this paper, we provide first evidence for a teleostean fish that the stress axis is influenced by leptin. RhLeptin attenuates stress axis activity in the common carp at multiple levels.

The restraint stressor that we applied resulted in mildly increased cortisol levels, as described previously [23,34]. The mild elevation of plasma cortisol values in pair-fed fish prevented discrimination of a rise in cortisol due to either netting or fasting. Clearly, pair-feeding is an important control and netting and fasting imposed only a mild stress to the fish. Unfortunately we do not (yet) have the tools to measure carp leptin protein levels, but in rats, an acute swim stress for ten minutes resulted in elevated leptin plasma levels [51].

Plasma glucose and NEFA levels indicate that this fish switches from a carbohydrate to a lipid based metabolism as glucose decreases and NEFA levels increase during fasting. The effect on glucose was potentially and partially confounded by the high levels of (hyperglycaemia inducing) cortisol after netting for 24 h; increased glucose levels after 24 h of restraint stress, compared to the 24 h non-fed control group, correlate positively with high cortisol levels in the stressed group. Despite this marked increase in plasma cortisol and glucose (a secondary stress parameter) levels, no changes in *leptin* expression due to stress were seen. The choice of (limited) sampling points (*i.e.* after 24 and 96 h of restraint) may have obscured changes in *leptin* expression. Moreover, cyprinid species have duplicate *leptin* genes as a result of an early, large-scale (possibly whole) genome duplication event around 300 million years ago [45,48]; these are called *leptin-a* and *leptin-b* [18,27]. The *leptin-a* paralogues (*leptin-a-I* and *leptin-a-II*) described in this paper [22] originated more recently as the result of an additional genome duplication event ± 16 million years ago in the common carp lineage [29]. One should predict the additional occurrence of two *leptin-b* paralogues in carp. Indeed, we recently identified two *leptin-b* paralogues in carp that we named *leptin-b-I* and *leptin-b-II*. As mentioned in the introduction, expression levels of *leptin-b* in liver were insignificant or too low to measure reliably. Importantly, we

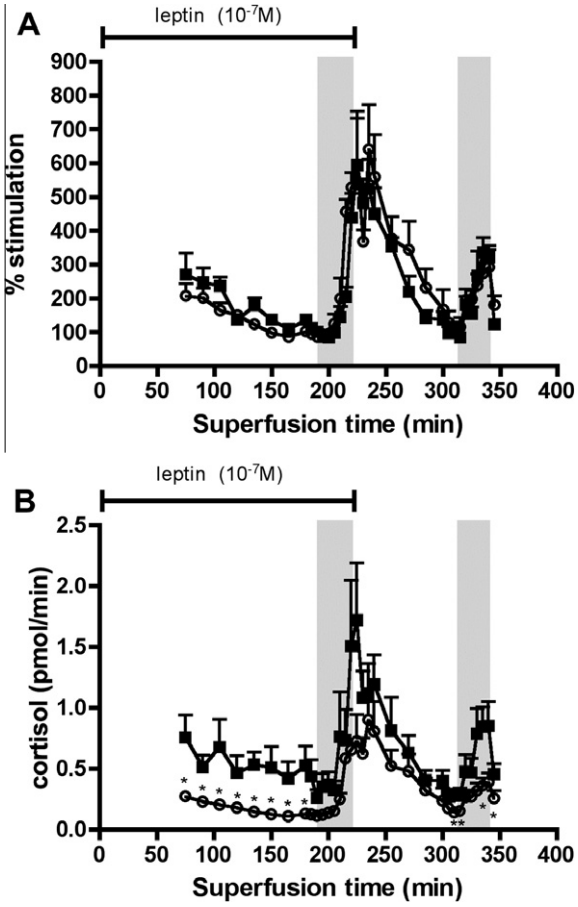

Fig. 5. In-vitro release of cortisol from head kidneys stimulated by 50 nM ACTH with (open dots) and without pre-conditioning with 100 nM leptin (closed squares). Values represent the percentage cortisol release relative to basal (A) and absolute amounts of cortisol release (B) by the head kidneys (pmol/min) ± S.E. ($n = 8$). Asterisks indicate significant differences between the pre-conditioned and the non-preconditioned group ($^*P < 0.05$).

observed a decrease in hepatic *leptin-a-II* expression after 96 h of fasting, an effect not seen in previous studies [22] when carp were fasted for up to 6 weeks. Such differential expression profile is in line with evolutionary conservation of two closely related genes resulting from a duplication event.

To study the effect of rhLeptin on various components of the stress axis we used a perifusion setup. Recombinant human leptin was used in these experiments, as a sufficient supply of homologous carp leptins was not available. Questions raised regarding the validity of the experiments are justified as human and carp leptins share no more than 25% amino acid identity. However, it should be noted that the tertiary structure of human and fish leptins is remarkably similar and suggestive that heterologous peptides may activate the same receptor [7,18,22,28]. Beyond that we feel that administration of human leptin to carp tissues is physiologically relevant, as human and *Xenopus* leptin (and these peptides display 35% amino acid identity) were shown to activate both the human and *Xenopus* leptin receptor (which also share a low 37% amino acid identity) with equal potencies [7].

When rhLeptin is administered to carp anterior pituitary gland cells (we have given evidence earlier [43] that our bisection does not result in contamination with proximal pituitary or pars intermedia cells), ACTH-release rapidly decreased (within 10 min) in a dose-dependent way. This effect was long lasting, as the ACTH release remained suppressed for more than an hour after application of rhLeptin was stopped. There are at least six isoforms of the leptin receptor in mammals (obese receptor; Ob-R), of which only the long form (Ob-Rb) evokes second messenger responses and biological effects [6]. In Atlantic salmon (*Salmo salar* L.) five leptin receptor mRNAs, splice variants from a single gene, are found [38] but, again, only one of the transcripts possesses both a transmembrane domain and the intracellular segments essential for signal transduction. The 'classical' view on leptin signalling includes the JAK/STAT pathway and the activation and/or inhibition of gene expression by STATs; however, alternative, signalling events of short duration have been suggested for leptin signalling [13]. The rapid and prolonged responses observed in our experiments do not preclude multiple signalling pathways. Short-term effects could impinge on a phospholipase C (PLC) pathway, more lasting responses to leptin will involve a JAK/STAT pathway [19].

In most fish species, ACTH release is under positive control of hypothalamic CRF [2,11,14,39,46]. In carp, CRF must be co-administered with dopamine to induce ACTH secretion [34]. Indeed, the CRF administration under DA- tonus increased ACTH release. When rhLeptin is co-administered from the start of the DA/CRF treatment, CRF-induced ACTH release is completely abolished.

Thus, both constitutive and regulated (CRF-induced) ACTH-secretion are attenuated by rhLeptin. The effects of rhLeptin on cortisol secretion are somewhat different. When head kidneys are exposed to rhLeptin, basal levels of (spontaneous) cortisol secretion decrease. The cortisol response to ACTH (expressed as percent change relative to basal levels) was not affected; clearly the absolute cortisol output of the interrenal cells is much lower than in the absence of rhLeptin. Whereas we cannot be sure what intracellular pathway(s) facilitate(s) the effects of rhLeptin on the interrenal cells, it is possible that rhLeptin interferes with the second messenger pathway of ACTH signalling (*i.e.* cAMP pathway), or that rhLeptin inhibits expression/activity of key steroidogenic enzymes that include 11β-hydroxylase and 21α-hydroxylase.

In recent studies, we found a marked and prolonged increase in hepatic *leptin-a-I* and *leptin-a-II* expression when carp are exposed to hypoxia [4] as well as to low ambient temperatures (Gorissen et al., in prep). In these cases, common carp (a truly eurytherm fish that naturally meets large variation in water oxygen levels and temperatures) reduce their energy expenditure to survive adverse conditions. Mounting a large and sustained stress response in these adaptations could be counterproductive, as fish then need to save precious fuel to cope with the environmental demand imposed on them. Leptin then may serve as a signal to downplay the stress axis, and by doing so may represent a crucial factor in adaptation processes when energy partitioning is critical. We stress the beauty of this eurytherm fish model to study fundamental roles of the pleiotropic cytokine leptin.

Acknowledgments

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