Acute and chronic leptin reduces food intake and body weight in goldfish (*Carassius auratus*)

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Abstract

The purpose of the present study was to elucidate the possible role of leptin in food intake and body weight regulation in goldfish. We examined the effects of i.c.v. or i.p. acute leptin administration on food intake in food-deprived goldfish at different time intervals post-injection (0–2, 2–8 and 0–8 h). Food intake was reduced by i.p. administered leptin (1 µg) at 8 h post-injection, without statistically significant differences after i.c.v. treatment. The present study shows for the first time in a teleost that chronic (10 days) leptin treatment (i.p.) reduces food intake, body weight gain, specific growth rate and food efficiency ratio. Moreover, lipid and carbohydrate metabolism seems to be regulated by leptin in fish.

Chronic leptin treatment increased lipid mobilization and carbohydrate storage as hepatic and muscle glycogen. Finally, leptin could mediate its actions on energy homeostasis in fish, at least in part, through interactions with hypothalamic catecholamines, since chronic leptin treatment reduced both hypothalamic noradrenergic and dopaminergic turnover without significant modifications in hypothalamic serotonergic and neuropeptide Y (NPY) systems. In summary, our results suggest that leptin can regulate feeding behaviour and body weight homeostasis in fish.

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Introduction

Leptin, a product of the obese (*ob*) gene, is a hormone secreted predominantly, although not exclusively, by adipose tissue (Zhang *et al.* 1994). Since its initial discovery in 1994, the biology of leptin has been most extensively studied in mammals, it having been described as a protein which exerts feeding, metabolic and neuro-endocrine effects (Muoio & Dohm 2002, Ahima & Osei 2004, Sahu 2004). One of the most important roles of leptin is its involvement in the regulation of energy balance by decreasing food intake and increasing energy expenditure (Ahima & Osei 2004, Sahu 2004).

Central and peripheral administration of leptin reduces food intake and body weight in rats (Seeley *et al.* 1996, Wetzler *et al.* 2004). Similar anorectic actions induced by leptin have also been described in other mammalian species, such as mice, pigs and monkeys (Sahu 2004). As in mammals, leptin also depresses food intake in chickens (Denbow *et al.* 2000) and wild birds (Löhms *et al.* 2003). Much less information is available on the biological role of leptin in ectotherm vertebrates. Previous reports on the possible physiological effects of leptin in fish have been published. Food intake was not modified by peripheral leptin in catfish under both fast and fed conditions (Silverstein & Plisetskaya 2000). Similarly, Baker and

co-workers (2000) reported that there were no differences in body weight, growth or energy stores after leptin implants were given over 2 weeks in fed and fasted Coho Salmon. Leptin injections (2 weeks) did not modify food intake and body weight in green sunfish (Londrville & Duvall 2002). However, Volkoff and co-workers (2003) have shown that acute administration of leptin significantly reduces food intake in goldfish.

Several studies indicate that feeding pattern alterations can modify leptin levels. In particular, fasting reduces leptin levels in plasma, adipose tissue and stomach, while overfeeding is accompanied by increased leptin concentrations in mammals (Ahima & Osei 2004). In fish, plasma leptin immunoreactive peptide was also reduced by 2 weeks fasting in green sunfish (Johnson *et al.* 2000) and burbot (Nieminen *et al.* 2003). Moreover, a positive correlation between blood leptin concentration and adiposity has been found in bluegill and white crappie (Johnson *et al.* 2000).

Feeding behaviour is finely tuned by a complex interplay of neurotransmitters, neuropeptides and hormones, and the hypothalamus plays an important role in such networks, which regulate energy homeostasis (de Pedro & Björnsson 2001, Volkoff *et al.* 2005). In mammals, it has been established that leptin most likely mediates its action on food intake and body weight regulation via

the hypothalamus, through a neural circuit comprising orexigenic and anorexigenic signals (Ahima & Osei 2004, Sahu 2004). As a rule, leptin inhibits appetite-stimulating pathways and stimulates appetite-suppressing pathways. Volkoff and co-workers (2003) have shown that leptin interacts with a number of neuropeptides to regulate feeding in fish. In particular, leptin decreases food intake in goldfish, in part by modulating orexigenic signals such as neuropeptide Y (NPY) and orexins, and through interaction with anorectic peptides, including cholecystokinin (CCK), cocaine and amphetamine regulated transcript (CART). On the other hand, interactions between leptin and monoamines in feeding regulation have also been suggested in mammals (Brunetti *et al.* 1999, Francis *et al.* 2004).

Leptin has been shown to have a wide repertoire of peripheral effects, mediated through the central nervous system or via a direct action on target tissues (Muoio & Dohm 2002, Veniant & Lebel 2003). Leptin appears to be a general metabolic hormone involved in many physiological processes, with adipocytes, liver, muscle and pancreas being the most important targets for this hormone (Frühbeck & Salvador 2000). Several effects of leptin on glucose metabolism have been reported in mammals, such as insulin inhibition, reduction of hepatic glycogenolysis and glucose production, increase of glucose uptake and glycogenesis in muscle (Frühbeck & Salvador 2000). Leptin is also known to act as a regulator of lipid reserves through changes in food intake, energy expenditure, fuel selection and lipid metabolism (Reidy & Weber 2000, Muoio & Dohm 2002). Exogenous leptin promotes fat metabolism by both inhibiting lipogenesis and stimulating lipolysis in mammals (Reidy & Weber 2000). Results in fish have also shown an increase in fat metabolism in response to leptin injections (Londraville & Duvall 2002).

In the present study, we analyzed in goldfish: the acute changes in food intake after central and peripheral injection of leptin; modifications in food intake and body weight throughout a course of chronic intraperitoneal injections of this hormone; the effects of chronic leptin on energetic reserves, particularly hepatic and muscle content of proteins, lipids and glycogen; and the possible interplay between leptin and some feeding regulators, such as NPY and monoamines, in feeding regulation.

Materials and Methods

Animals

Goldfish were obtained from a commercial supplier and were maintained under 12 L:12D photoperiod and $21 \pm 2^\circ\text{C}$ water temperature in aquaria (50 l) with a constant flow of filtered water. Fish were fed once daily with a 1% body weight (bw) ration of floating pellets (Sera Biogram, Heinsberg, Germany) at 1000–1100 h. Animals were acclimated to these conditions for at least 15 days

prior to experimental use, showing a normal feeding pattern during this acclimation period. The care and use of animals were in accordance with the UFAW handbook on the care and management of laboratory animals and approved by the local ethics committee.

Hormone administration

Recombinant human leptin (Sigma Chemical Co.) was dissolved in teleost saline (20 mg Na_2CO_3 per 100 ml of 0.6% NaCl). Fish were anesthetized in water containing tricaine methanesulphonate (MS-222, 1:10 000). Immediately after loss of equilibrium, fish were injected between 1030 and 1130 h in both acute and chronic treatments. The i.p. injections were performed with a 1 ml syringe and 0.3 mm Microlance needle (Lab-center, Madrid, Spain) close to the ventral midline posterior to the pelvic fins. The i.c.v. injections were carried out using a 0.3 mm Microlance needle connected to a 5 μl Hamilton microsyringe (Lab-center) with an 18 Venocath cannula (Lab-center). Injections were performed freehand through the central junction between the parietal and frontal bones. The i.p. and i.c.v. procedure and accuracy of injection placement into the ventricular regions of the fish brain were previously established (de Pedro *et al.* 1993).

Experimental design

Food intake after acute i.p. and i.c.v. leptin administration Food-deprived (48 h) goldfish (11.7 ± 0.12 g bw; $n=7-9/\text{group}$) were either peripherally or centrally injected. In the peripheral administration, fish were i.p.-injected with 10 μl saline/g bw alone (control group) or containing leptin (experimental groups) at doses of 0.1, 0.33, 1 and 3.3 $\mu\text{g/g}$ bw. In the central administration, animals were i.c.v. – injected with either 1 μl teleost saline alone (control group) or containing leptin (experimental groups) at doses of 0.1, 0.33, 1, 3.3 and 10 μg .

Fish recovered equilibrium and normal swimming activity in anaesthetic-free water within 1–2 min after treatments. Immediately, individual goldfish were transferred to 5 l aquaria, and 10 min after the injection received pre-weighed food in excess (5% body weight). Food intake (FI) was measured at 2 and 8 h post-injection, and it was calculated as follows:

$\text{FI} = \text{Wi} - (\text{Wf} \times \text{F})$ where Wi = initial dry food weight, Wf = remaining dry food weight and F = correction factor (de Pedro *et al.* 1993).

Effect of chronic leptin i.p. administration on feeding, body weight, metabolic reserves and monoamines Two groups of goldfish (8.74 ± 0.22 g bw) were i.p.-injected for 10 days with 10 μl teleost saline/g bw alone ($n=9$) or containing 1 $\mu\text{g/g}$ bw of human leptin ($n=10$). Daily food intake was individually measured

at 8 h post-injection throughout the experimental period. Body weight was recorded daily. At the end of the experiment, fish were weighed and measured after 24 h post-injection and under food deprived conditions. Blood was withdrawn by cardiac puncture using a 1 ml sterile plastic heparinized syringe and a 0.5 mm Microlance needle, and plasma samples were stored at -80°C until used. Fish were killed by decapitation and total liver was carefully dissected and samples of muscle were removed, weighed and frozen on solid CO_2 . The hypothalamus were rapidly removed and frozen on solid CO_2 . All these tissue samples were stored at -80°C until analysis.

Analytical procedures

Biometric parameters Weight increase (body weight gain) and specific growth rate (SGR) were determined. SGR was calculated as follows: $\text{SGR} = [(\ln W_f - \ln W_i) / t] \times 100$, where W_i and W_f are initial and final body weights, respectively and t is the time interval (in days) between W_i and W_f measurements. Three biometric indexes were also calculated: nutritional index (or condition factor), $\text{NI} = \text{final body weight} \times 100 / [\text{length (cm)}]^3$; hepatosomatic index, $\text{HSI} = \text{liver weight} \times 100 / \text{body weight}$; and food efficiency ratio, $\text{FE} = \text{g body weight gain/g feed intake}$.

Plasma biochemistry Glucose (Glucose Trinder) and triglycerides (GPO-Trinder) were determined using enzymatic/colorimetric methods by means of commercial kits (Sigma Diagnostics).

Metabolic resources Protein content in liver and muscle was determined as described by the method of Lowry *et al.* (1951), using bovine serum albumin as the standard. Total lipids in liver and muscle samples were extracted with chloroform:methanol (2:1) according to Folch's procedure (Folch *et al.* 1957), evaporated to dryness and reconstituted in dioxane at 100°C . Total lipid content was determined by spectrophotometry (505 nm) using triolein as standard. Liver and muscle glycogen content was quantified by spectrophotometry (Dubois *et al.* 1956) after extraction with ethanol and previous digestion with KOH (Montgomery 1957).

Hypothalamic monoamines The monoamines analyzed were epinephrine (E), norepinephrine (NE) and its main metabolite (3-methoxy-4-hydroxyphenylglycol (MHPG)), dopamine (DA) and its metabolite (dihydroxyphenylacetic acid (DOPAC)), and serotonin (5-HT) and its metabolite (5-hydroxyindol-3-acetic acid (5-HIAA)). Hypothalamic content of all these compounds was quantified by high-performance liquid chromatography (HPLC) with coulometric detection (Coulchem II; ESA, Bedford, USA), as previously described (de Pedro *et al.* 2001). Briefly, half of the hypothalamus was homogenized

by sonication in 125 μl cold 0.2 N perchloric acid containing 0.2 mM sodium bisulphite, 0.4 mM EDTA and 100 nM isoproterenol as internal standard. The homogenate was centrifuged (16060 g for 1 min) and supernatant injected into the HPLC system. The HPLC system consisted of a Waters pump (Model 590; Waters, Madrid, Spain), a pulse dampener and an automatic injector (Agilent Technologies, Madrid, Spain) with a C18 reversed phase column (125x4.6 mm ID, 5 μm particle size). Electrode voltages were +50 and +300 mV for analytical cells n° 1 and 2, respectively. The mobile phase (flow rate = 1 ml/min) consisted of 10 mM H_3PO_4 , 0.1 mM EDTA, 0.4 mM sodium octanesulphonic acid and 3.5% acetonitrile (pH 2.84).

Protein content in the hypothalamus was determined as described by the method of Lowry *et al.* (1951). Monoamine content in the samples was calculated as the area under the peaks, and expressed as ng/mg proteins (ng/mg prot).

Hypothalamic NPY Half of the hypothalamus was incubated for 10 min at 100°C in 125 μl of 2 M acetic acid containing 200 $\mu\text{g/ml}$ aprotinin. It was then sonicated and the homogenate was centrifuged (16000 g for 3 min). The supernatant was used to determine hypothalamic content of NPY by radioimmunoassay using a commercial kit (Bachem S-2029; Peninsula Laboratories Europe, Merseyside, England). The lower and upper limits of sensitivity of the assay are 1 and 128 pg/tube. The intra- and interassay coefficients of variation were 3.64% and 6.85% (2 pg/tube) and 9.36% and 14.77% (64 pg/tube), respectively. Hypothalamic samples alone or with added synthetic NPY were assayed to determine the efficiency of the RIA for detection of goldfish NPY. Linear regression between the amount of hormone found and amount of hormone expected showed a slope of nearly 1 and recovery was 100.22%. Validation was assessed by testing those displacement curves obtained with serial dilutions of hypothalamic samples with NPY standard curve. Figure 1 shows that serial dilutions of hypothalamic samples gave parallel displacement with respect to NPY standard curve. No significant differences were found between slopes after logit-log data transformation (standard curve: $b = -45.01 \pm 4.09$, serial dilutions of hypothalamic samples: $b = -46.35 \pm 5.24$, $P = 0.842$).

Statistical analysis

Data from acute leptin injection experiments were analyzed through an analysis of variance (ANOVA) test followed by the Student-Newman-Keuls (SNK) multiple range test for multigroup comparisons. In the chronic experiment, a Student's t -test was used to ascertain statistical differences between saline- and leptin-injected fish at the end of the treatment. Food intake and body weight gain data obtained every day during chronic treatment

Figure 1 Comparison of NPY standard curve (solid line) with serial dilutions of hypothalamic samples (dashed line). Each point represents the average of duplicate determinations. B/Bo=binding/maximum binding.

were analyzed by one-way ANOVA with repeated measures (Huynh-Feldt). A probability level of $P < 0.05$ was considered statistically significant.

Results

Figure 2 shows food intake during discrete and cumulative intervals after acute (a) i.p. and (b) i.c.v. injection of either vehicle or leptin (0.1, 0.33, 1 and 3.3 $\mu\text{g/g}$ bw for i.p. treatment; 0.1, 0.33, 1, 3.3 and 10 μg for i.c.v. treatment). Food intake was significantly ($P < 0.05$) reduced relative to the control group in intervals 2–8 and 0–8 h after i.p. injection of 1 μg leptin, but not during the discrete interval 0–2 h. i.c.v.-administered leptin did not significantly reduce feeding, although a decreasing trend in cumulative food intake was observed at 8 h with the 1 μg doses.

The chronic effects of leptin on food intake and body weight gain in goldfish are presented in Figure 3. i.p.-administered leptin at a dose of 1 $\mu\text{g/g}$ bw significantly suppressed spontaneous feeding throughout the experimental period (10 days), except on the 5th and 6th day after starting hormone administration (Fig. 3a). The leptin treatment for 10 days significantly reduced body weight gain (Fig. 3b) compared with the saline controls, and this reduction was observed from the first day of treatment. After chronic leptin treatment, both food efficiency ratio and specific growth rate in leptin-treated fish were significantly lower ($P < 0.005$) than that of saline-treated fish (Table 1). No significant differences in nutritional or hepatosomatic indexes were observed after leptin administration (Table 1).

Glucose (saline-injected: 107.8 ± 14.81 mg/dl, leptin-injected: 69.46 ± 6.5 mg/dl) and triglycerides (saline-injected: 267.9 ± 33.7 mg/dl, leptin-injected: 167.8 ± 20.6 mg/dl) plasma levels were significantly ($P < 0.05$) reduced by chronic i.p. leptin administration in goldfish.

Figure 2 Food intake (mg) after (a) i.p. injection of leptin at the doses of 0, 0.1, 0.33, 1 and 3.3 $\mu\text{g/g}$ bw at 2 and 8 h post-injection and (b) i.c.v. administration of 1 μl saline alone or containing leptin at the doses of 0.1, 0.33, 1, 3.3 and 10 μg in goldfish ($n = 7$ –9/group). Data are expressed as mean $\pm$ S.E.M. * $P < 0.05$ compared with control group.

Figure 4 summarizes the results obtained regarding the role of leptin on metabolic reserves. As can be observed (Fig. 4a), hepatic and muscle content of glycogen was significantly increased ($P < 0.05$) in leptin-injected fish compared with the control group. After 10 days of leptin treatment, total lipids were significantly reduced (two fold) in liver, but not in muscle (Fig. 4b). Leptin did not alter total protein content in either liver or muscle (Fig. 4c).

The hypothalamic content of NPY, monoamines and their metabolites after chronic treatment with leptin (1 $\mu\text{g/g}$ bw) in goldfish is presented in Table 2. There were no significant modifications in the hypothalamic content of NPY after chronic leptin treatment. The MHPG/NE ratio was reduced in leptin-treated compared with saline-treated fish, but no statistically significant changes in hypothalamic content of both NE and MHPG were found. Leptin treatment reduced ($P < 0.05$) hypothalamic content of DOPAC, which subsequently reduced the DOPAC/DA ratio. Both 5-HT and 5-HIAA are part

Figure 3 Effect of i.p. administration of leptin (1 µg/g bw) during 10 days on (a) food intake and (b) body weight gain in goldfish ($n=9-10/\text{group}$). Data are expressed as mean $\pm$ S.E.M. * $P<0.05$, ** $P<0.01$, *** $P<0.001$ compared with saline in the same day.

of the hypothalamic content, so the 5-HIAA/5-HT ratio was not significantly modified by leptin treatment.

Discussion

The present findings show a clear effect of exogenous leptin on food intake and body weight in goldfish.

Table 1 Effects of i.p. chronic administration of leptin (1 µg/g bw) on different biometric parameters in goldfish after 10 days of treatment

	Saline	Leptin
Mean daily food intake (mg)	306.5 $\pm$ 9.86	216.5 $\pm$ 7.06*
Body weight gain (%)	7.39 $\pm$ 0.86	2.43 $\pm$ 1.22†
Specific growth rate (%/day)	0.711 $\pm$ 0.08	0.234 $\pm$ 0.12†
Food efficiency ratio	0.319 $\pm$ 0.04	0.065 $\pm$ 0.04†
Nutritional index	3.30 $\pm$ 0.06	3.19 $\pm$ 0.07
Hepatosomatic index (%)	3.92 $\pm$ 0.44	4.61 $\pm$ 0.29

Data are presented as mean $\pm$ S.E.M. Saline ($n=9$); Leptin ($n=10$). * $P<0.001$; † $P<0.005$.

Peripheral leptin significantly reduced food intake after both acute and chronic treatments, indicating that tolerance did not develop, at least not during the first 10 days. Data from the acute experiment confirm previous reports in goldfish, where central and peripheral leptin at different doses and time intervals post-injection also decreases food intake (Volkoff *et al.* 2003). All these data also corroborate in fish the anorectic action of leptin in mammals, birds (Májáčová *et al.* 2004) and reptiles (Niewiarowski *et al.* 2000), suggesting that feeding regulation by leptin could be one example of a highly conserved physiological process in vertebrate evolution. On the other hand, previous reports in mammals and fish have indicated that leptin is much less potent in reducing food intake after peripheral than central injection, suggesting the hypothalamus as the main target for leptin action. In the present study, however, i.p. leptin appears to be more effective than i.c.v. injection. Recent work in mammals (Berthoud 2005, Peters *et al.* 2005) has shown that leptin is also secreted from gastric mucosa, and one of the major physiological roles of leptin in regulating food intake appears to occur directly in the gut, which supports our results. In fact, Peters and co-workers have suggested that leptin exerts multiple actions in regulating food intake and body weight, with gastric leptin acting at the gastrointestinal level in combination with leptin from adipose tissue acting on hypothalamic sites.

In the present study, chronic treatment with mammalian leptin reduced body weight gain in goldfish. The leptin-induced food intake reduction and lower food conversion efficiency observed in the present study explain such lower body weight gain in leptin-treated goldfish. Weight loss after leptin administration in mammals is associated with both a decrease in food intake and an increase in thermogenesis and activity level (Wang *et al.* 1999, Wetzler *et al.* 2004). To date, it is unknown if leptin modifies activity in fish, which could also contribute to body weight reduction.

Decreases in plasma glucose and triglycerides after leptin infusion in goldfish are in agreement with findings in mammals (Wang *et al.* 1999, Ramsay *et al.* 2004). This glycaemia decrease may be associated with reduced hepatic glucose production and/or increased glucose utilization, as has been described in mammals (Wang *et al.* 1999). An increase found in the present study in the storage of glucose as either hepatic or muscle glycogen suggests that other mechanisms could contribute to hypoglycaemia in goldfish. In fact, a partial inhibition of hepatic glycogenolysis by leptin in mammals has been reported (Frühbeck & Salvador 2000), which probably leads to preservation of hepatic glycogen stores, in agreement with the increase in liver glycogen observed in goldfish.

To date, this is the first report studying possible leptin actions on lipids in fish. A significant reduction in hepatic content of total lipids after leptin administration for

Figure 4 Effect of i.p. administration of leptin (1 µg/g bw) during 10 days on liver and muscle content of (a) glycogen, (b) lipids and (c) proteins in goldfish ($n=9-10/\text{group}$). Data are expressed as mean $\pm$ S.E.M. * $P<0.05$.

10 days in goldfish is in accordance with the general role of peripheral leptin increasing lipolysis in mammals (Reidy & Weber 2000). Our data support the hypothesis that the lipolytic action of leptin is highly conserved throughout phylogeny (Frühbeck *et al.* 2001, Ramsay *et al.* 2004).

Leptin actions on energy homeostasis in goldfish could be mediated, at least in part, by interactions with hypothalamic catecholamines, since chronic leptin treatment reduced both hypothalamic noradrenergic and dopaminergic turnover without significant modifications in the serotonergic system. Our data support previous studies in rats, which demonstrated that part of the anorectic activity of leptin could be mediated by inhibition of noradrenergic and dopaminergic fibres in the

hypothalamus (Brunetti *et al.* 1999). Particularly, leptin could probably reduce food intake by decreasing noradrenergic activity in fish hypothalamus, keeping in mind that NE stimulates food intake in fish (de Pedro & Björnsson 2001, de Pedro *et al.* 2001). These results are consistent with findings in mammals, where NE appears to be involved in feeding regulation by leptin (Francis *et al.* 2004). Our findings show that leptin did not modify either hypothalamic serotonin or its metabolite 5-HIAA, which seems to exclude the possibility of some involvement of the serotonergic pathway in the anorectic effect of leptin in fish. This has also been observed to be the case in rats (Orlando *et al.* 2001). Nevertheless, it is possible that alterations in catecholamines following leptin administration would be secondary to leptin's actions on food

Table 2 Effects of i.p. chronic administration of leptin (1 µg/g bw) on hypothalamic content of NPY, monoamines and their metabolites in goldfish after 10 days of treatment

	Saline	Leptin
NPY (ng/hyp)	4.87 ± 0.4	5.44 ± 0.37
Epinephrine (ng/mg prot)	0.105 ± 0.02	0.115 ± 0.01
Norepinephrine (ng/mg prot)	9.25 ± 1.07	9.37 ± 0.91
MHPG (ng/mg prot)	18.52 ± 1.20	15.46 ± 1.36
MHPG/NE (%)	206.3 ± 14.1	168.9 ± 9.82*
Dopamine (ng/mg prot)	9.87 ± 2.04	10.77 ± 1.95
DOPAC (ng/mg prot)	0.225 ± 0.03	0.151 ± 0.01*
DOPAC/DA (%)	2.51 ± 0.19	1.56 ± 0.21*
Serotonin (ng/mg prot)	17.37 ± 3.10	18.38 ± 2.72
5-HIAA (ng/mg prot)	0.809 ± 0.12	0.972 ± 0.09
5-HIAA/5-HT (%)	4.89 ± 0.77	5.96 ± 0.59

Data are presented as mean ± S.E.M. Saline (n=9); Leptin (n=10). *P<0.05.

intake and body weight. In fact, fasting for 7 days activated the noradrenergic system and decreased the dopaminergic system in goldfish hypothalamus (de Pedro *et al.* 2001). Therefore, the reduction in dopaminergic turnover observed in the present study could be a response to leptin-induced feeding inhibition and not solely due to a direct effect of leptin treatment.

NPY is one of the most potent orexigenic signals in goldfish (Lopez Patiño *et al.* 1999), and previous reports have indicated that leptin reduces feeding and NPY mRNA expression in the telencephalon and hypothalamus (Volkoff *et al.* 2003) in parallel. Moreover, in goldfish, leptin modulates the orexigenic effects of exogenous NPY at doses that did not influence food intake when given alone (Volkoff *et al.* 2003). However, in our study, NPY hypothalamic content was unaffected by leptin and, as a result, the inhibition of food intake and body weight by chronic leptin in goldfish is not likely to result from NPY alterations. The apparent discrepancy between our results and previous findings might simply be due to differences in experimental design (chronic *vs.* acute administration, different fish size, different leptin doses, and determination of immunoreactive NPY *vs.* NPY mRNA expression, among others). On the other hand, it could be indicative of a possible development of leptin resistance in NPY neurones, which would explain the lack of effect of leptin on NPY hypothalamic content after repeated injections, as has also been described in some chronic studies in rats (Sahu 2004).

In conclusion, leptin could be acting as a peripheral regulator of feeding and body weight in goldfish, which supports the idea that it has a role as a signal of nutritive state in fish and implies a high conservation of leptin actions in energy balance throughout phylogeny.

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