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Neuropeptide Y has a stimulatory action on feeding behavior in goldfish (*Carassius auratus*)

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Abstract

The purpose of the present study was to elucidate the possible role of neuropeptide Y (NPY) in the feeding regulation in fish. We examined the effects of intracerebroventricular (i.c.v.) or intraperitoneal (i.p.) neuropeptide Y administration on food intake in satiated goldfish, at different time intervals postinjection (0–2, 2–8 and 0–8 h). Food intake was significantly increased by i.c.v. administered neuropeptide Y (1 μg) at 2 h postinjection, while no significant differences in food intake were observed after i.p. treatment. The neuropeptide Y receptor antagonist, neuropeptide Y-(27–36), totally counteracted the stimulatory action of neuropeptide Y on feeding. The possible involvement of neuropeptide Y in the eating behavior evoked by food deprivation has been investigated. Food deprivation by either 24 or 72 h significantly increased feeding, and the neuropeptide Y receptor antagonist attenuated such feeding stimulation. From our findings, we suggest, first, that neuropeptide Y is involved in feeding central regulation in goldfish, acting via specific neuropeptide Y receptors, and second, that hypothalamic neuropeptide Y would be released in response to food deprivation, contributing to generate the consequent eating behavior stimulation in *Carassius auratus*.

Keywords: Neuropeptide Y; Feeding; Food deprivation; Antagonism; (Goldfish)

1. Introduction

Neuropeptide Y (NPY) is a 36-amino acid peptide that was first isolated from porcine brain (Tatemoto, 1982). Subsequently, the neuropeptide Y amino-acid sequence was determined in other mammalian species, and also in some birds, reptiles, amphibians and fish. Twenty-two positions are identical in all known neuropeptide Y sequences, indicating a remarkable degree of neuropeptide Y sequence homology throughout phylogeny (Larhammar, 1996; Larhammar et al., 1993, 1997).

Neuropeptide Y is widely distributed through central and peripheral nervous system of mammals (Balasubramaniam, 1997). In the central nervous system (CNS), this peptide is particularly dense in the paraventricular nucleus of the hypothalamus (Hendry, 1993), where neuropeptide Y has been involved in the feeding regulation (Stanley and Leibowitz, 1984, 1985). Neuropeptide Y also exits in the CNS of fish. Immunohistochemical studies have identified neuropeptide Y-like immunoreactivity in the brains of several fish species (*Carassius auratus*, Pontet et al., 1989; *Oncorhynchus mykiis*, Danger et al., 1991; *Acipenser transmontanus*, Chiba and Honma, 1994; *Protopterus annectens*, Vallarino et al., 1995; *Polypterus senegalus*, Chiba, 1997). This widespread distribution of neuropeptide Y in vertebrates implies that this peptide can be involved in several physiological functions. In fact, neuroepeptide Y has been involved in cardiovascular control, anxiety, sexual behavior, neuroendocrine secretion and feeding in mammals (Allen and Bloom, 1986; Balasubramaniam, 1997). To date, the only known role of neuropeptide Y in fish is as a neurohormone in controlling pituitary hormone secretions, e.g., as stimulator of growth hormone and gonadotropines (Peng et al., 1990).

One of the most studied actions of neuropeptide Y in mammals has been the possible role of neuropeptide Y on

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feeding regulation. In 1984, it was described that neuropeptide Y increases food intake after central administration in rats (Clark et al., 1984; Levine and Morley, 1984; Stanley and Leibowitz, 1984). Subsequent to these first reports, several studies have also shown that neuropeptide Y stimulates feeding after central administration in a wide range of situations and vertebrate species (Leibowitz, 1995; Levine and Billington, 1997).

Further, it has also been demonstrated that feeding pattern alterations can produce modifications in the brain neuropeptide Y content (Leibowitz, 1995). Particularly, food deprivation induces an increase in both neuropeptide Y content (rat, Yoshihara et al., 1996), and neuropeptide Y gene expression in hypothalamus (rat, Schwartz et al., 1993; hamster, Mercer et al., 1996). Recently, greater neuropeptide Y-like gene expression in preoptic area of the hypothalamus was also found with fasting in teleosts (Silverstein et al., 1998).

It is known the involvement of certain neuropeptides, such as corticotropin-releasing factor (de Pedro et al., 1993), bombesin (Himick and Peter, 1994a), cholecystokinin (Himick and Peter, 1994b), β-endorphin (de Pedro et al., 1995a) and galanin (de Pedro et al., 1995b) in the modulation of feeding in fish. However, to date, there are no studies about the role of neuropeptide Y, the most powerful known peptide stimulant of eating behavior in mammals, on feeding regulation in fish. The purpose of the present work was to study the actions of neuropeptide Y on food intake in goldfish (*C. auratus*). First, we determined the effects of the neuropeptide Y administration via the CNS or peripherally on food intake in goldfish. Second, we tested if the general antagonist of neuropeptide Y receptors, [D-Tyr^{27,36}, D-Thr^{32}] neuropeptide Y-(27–36), would block the neuropeptide Y effect on food intake. Finally, we studied the possible involvement of neuropeptide Y in the eating behavior evoked by starvation.

2. Materials and methods

2.1. Animals

Goldfish (*C. auratus*), 7.86 ± 0.41 g body weight (bw), were obtained from a commercial supplier in Madrid. They were maintained under 12 L:12 D photoperiod and 21 ± 2°C water temperature in 50 l aquaria, with a constant flow of filtered water. Fish were fed once daily with a 1% bw ration of floating pellets (Sera Biogram) at 1000–1100 h. Animals were acclimated to these conditions for at least 15 days prior to the experimental use, showing a normal feeding pattern during this acclimation period.

2.2. Drugs and treatment

Porcine neuropeptide Y (Sigma, Spain) and [D-Tyr^{27,36}, D-Thr^{32}] neuropeptide Y-(27–36) (RBI, USA) were dissolved in teleost saline (20 mg Na_2CO_3/100 ml of 0.6% NaCl). The intracerebroventricular (i.c.v.) injections were carried out using a 0.3 mm Microlance needle connected to a 5 μl Hamilton microsyringe with a 18 Venocath cannula. The intraperitoneal (i.p.) injections were performed with a 1 ml syringe and 0.3 mm Microlance needle, close to the ventral midline posterior to the pelvic fins. The i.c.v. and i.p. procedure and the accuracy of injection placement into the ventricular regions of the fish brain was previously established (de Pedro et al., 1993).

2.3. Experimental design

Daily food ration was available 1 h before the injections to ensure that fish were satiated at the time of feeding test, except for the starvation experiment. Animals were anesthesized in water containing tricaine methanesulphonate (MS-222, 1:10,000). Immediately after loss of equilibrium, fish were injected between 1030 and 1130 h.

2.3.1. Experiment 1. Food intake after i.c.v. administration of neuropeptide Y

Animals were injected with either 1 μl teleost saline alone (control group) or containing neuropeptide Y (experimental groups) at doses of 0.033 ($n = 8$), 0.1 ($n = 9$), 0.33 ($n = 8$), 1 ($n = 9$) and 3.3 ($n = 8$) μg.

2.3.2. Experiment 2. Food intake after i.p. administration of neuropeptide Y

Fish were divided into three groups ($n = 8$/group): (a) one control group, i.p. injected with 10 μl saline/g bw, and (b) two neuropeptide Y groups, i.p. injected with neuropeptide Y (0.1 and 0.33 μg/g bw, respectively).

2.3.3. Experiment 3. Effect of i.c.v. administration of [D-Tyr^{27,36}, D-Thr^{32}] neuropeptide Y-(27–36) on neuropeptide Y-induced feeding

Four groups ($n = 10$/group) of fish received two sequential i.c.v. injections, separated by 10 min: (a) control group: two injections of 1 μl saline; (b) neuropeptide Y group: saline followed by neuropeptide Y (1 μg) injection; (c) neuropeptide Y-(27–36) group: 1 μl neuropeptide Y-(27–36) (5 μg) injection followed by 1 μl saline injection; (d) neuropeptide Y-(27–36) + neuropeptide Y group: antagonist (5 μg) injection followed by neuropeptide Y (1 μg) injection.

2.3.4. Experiment 4. Neuropeptide Y as mediator of food intake stimulation by starvation

Two different experiments were carried out. In Experiment 4.1, 30 goldfish were divided in the following three groups ($n = 10$/group): (a) control group (F): fish were daily fed (1% bw); (b) 24 h starved group (24 SV): fish

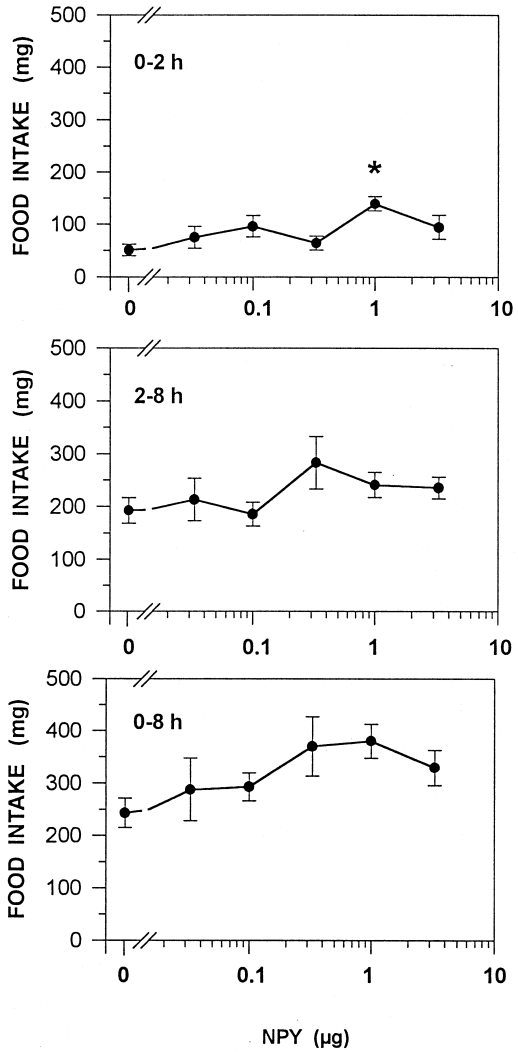

Fig. 1. Food intake (mg) after i.c.v. administration of 1 μl saline alone ($n = 9$) or containing neuropeptide Y at doses 0.033 ($n = 8$), 0.1 ($n = 9$), 0.33 ($n = 8$), 1 ($n = 9$) y 3.33 ($n = 8$) μg at 0–2 (top), 2–8 (middle) and 0–8 (bottom) h postinjection, in goldfish (*C. auratus*). The *x*-axis represents absolute doses of neuropeptide Y (μg) in all graphs. Data are expressed as mean ± S.E.M. $^{*}P < 0.05$ compared to control group (Student–Newman–Keuls test).

were 24 h food-deprived; (c) 72 h starved group (72 SV): fish were 72 h food-deprived.

In Experiment 4.2, twenty four fish ($n = 8$/group) were divided in the following three groups: (a) fed + saline group (F + SAL): fish received food (1% bw) daily and were i.c.v. injected with 1 μl saline solution; (b) 24 h starved + saline group (24 SV + SAL): goldfish were i.c.v. injected with 1 μl saline after 24 h fasting; (c) 24 h starved + antagonist group [24 SV + neuropeptide Y-(27–36)]: goldfish were i.c.v. injected with 5 μg neuropeptide Y-(27–36) after 24 h food deprivation.

In all experiments, fish recovered equilibrium and normal swimming activity in anesthesic-free water within 1–2 min after treatments. Immediately, individual goldfish were transferred to 5 l aquaria, and 10 min after the last injection received preweighed food in excess (5% bw). Food intake (FI) was measured at 2 (Experiments 1, 2, 3 and 4) and 8 (Experiments 1 and 2) h postinjection, and it was calculated as follows: $FI = W_i - (W_f \times F)$, where W_i = initial dry food weight, W_f = remaining dry food weight, and F = correction factor. F was calculated to evaluate the effect of water dissolution on food pellets during the feeding time, and represents the reduction in food weight after food remains 2 ($F = 1.19 \pm 0.0062$, $n = 20$) and 6 ($F = 1.27 \pm 0.0036$, $n = 20$) h into the aquaria.

2.4. Statistical analysis

Data were analyzed by an analysis of variance (ANOVA) test followed by Student–Newman–Keuls multiple range test for multigroup comparisons. A probability level of $P < 0.05$ was considered statistically significant.

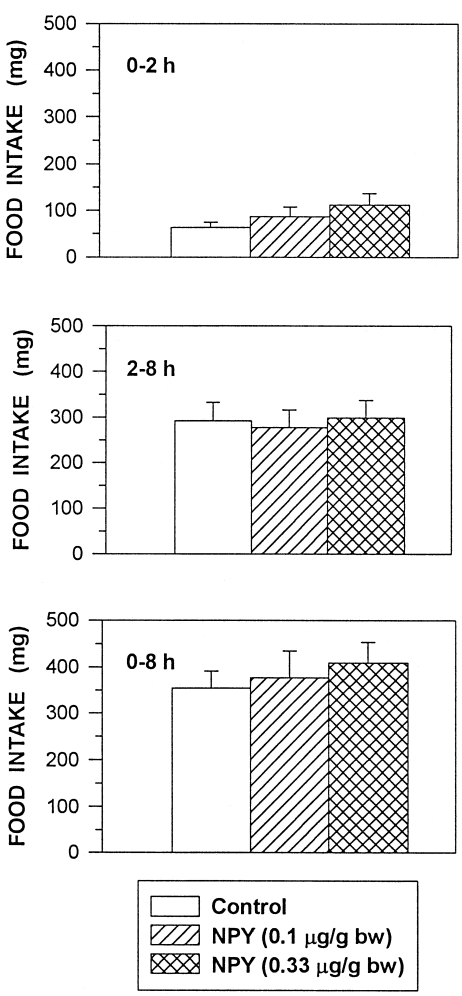

Fig. 2. Effect of i.p. administration of neuropeptide Y (0.1 and 0.33 μg/g bw) on food intake (mg) in goldfish (*C. auratus*) at 0–2 (top), 2–8 (middle) and 0–8 (bottom) h postinjection ($n = 8$/group). Data are expressed as mean + S.E.M.

3. Results

Fig. 1 shows food intake during discrete and cumulative intervals after i.c.v. injection of either neuropeptide Y (0.033; 0.1; 0.33; 1 and 3.3 μg) or vehicle. FI was significantly increased (twofold) by 1 μg of neuropeptide Y respect to the control group at 2 h postinjection ($F(5,41) = 3.44$, $P < 0.05$). During the discrete interval 2–8 h, none of the doses tested significantly modified food intake ($F(5,43) = 1.30$, $P > 0.05$). An increasing trend of cumulative food intake was observed at 8 h, although this feeding rate stimulation was not statistically significant ($F(5,41) = 1.71$, $P > 0.05$).

The effects of i.p. administration of neuropeptide Y (0.1 and 0.33 μg/g bw) are presented in Fig. 2. No significant differences in food intake after neuropeptide Y treatment were observed at any of the studied time intervals ($F(2,20) = 1.5$, $P > 0.05$).

The i.c.v. administration of [D-Tyr$^{27-36}$, D-Thr^{32}] neuropeptide Y-(27–36) (5 μg) by itself did not significantly alter food intake in goldfish (Fig. 3). This same doses immediately administered before neuropeptide Y totally counteracted the stimulatory action of the neuropeptide Y on eating in goldfish ($F(3,36) = 5.95$, $P < 0.01$).

Fig. 4 summarizes the results obtained from Experiment 4 about the role of neuropeptide Y as mediator of eating induced by food deprivation. As it can be observed (Fig. 4a), the food deprivation by either 24 or 72 h significantly increased feeding ($F(2,27) = 8.36$, $P < 0.01$). Such increases were similar after 24 or 72 h of starvation and represented around 111–121%, respectively, in relation to the control group. These increases were similar to the rise (138%) induced by the i.c.v. administration of 1 μg neuropeptide Y (Fig. 4b). The feeding stimulation ($P < 0.01$) induced by 24 h starvation was partially reversed by the i.c.v. administration of neuropeptide Y receptor general antagonist [D-Tyr$^{27-36}$, D-Thr^{32}] neuropeptide Y-(27–36), ($F(2,23) = 7.46$, $P < 0.05$) (Fig. 4c).

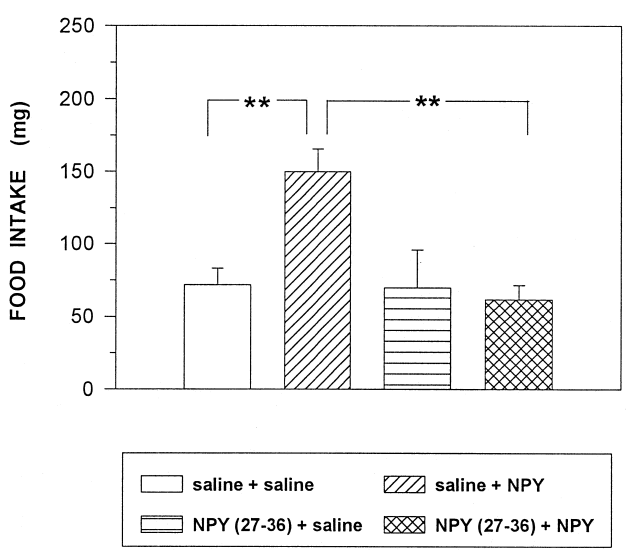

Fig. 3. Food intake (mg) after i.c.v. administration of 1 μl saline (control); 1 μg of neuropeptide Y; 5 μg of neuropeptide Y-(27–36); and both, 5 μg of neuropeptide Y-(27–36) + 1 μg of neuropeptide Y at 2 h postinjection in goldfish (*C. auratus*) ($n = 10$/group). Data are expressed as mean + S.E.M. $^{**}P < 0.01$ (Student–Newman–Keuls test).

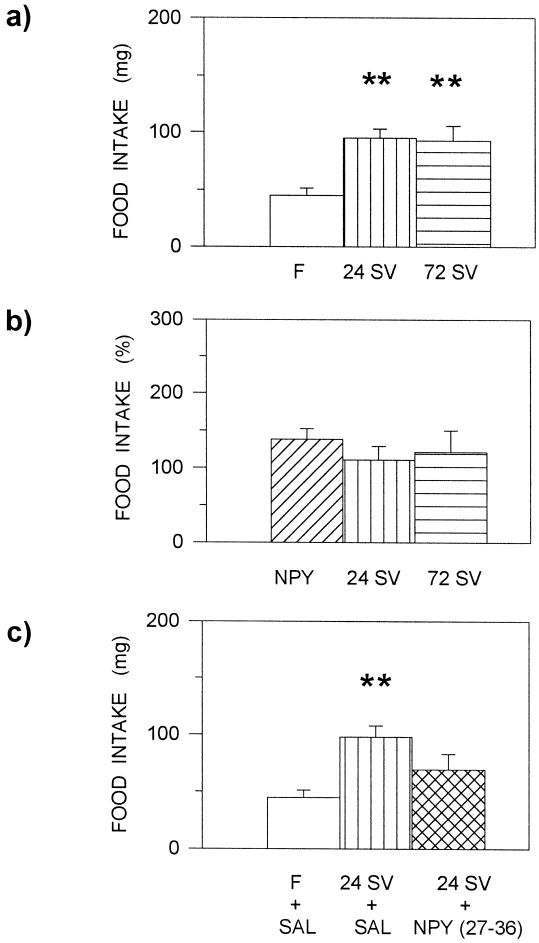

Fig. 4. (a) Food intake (mg) in daily fed (F) and starved goldfish by 24 (24 SV) and 72 (72 SV) h at 2 h of food exposure ($n = 10$/group). (b) Effect of i.c.v. injection of neuropeptide Y (1 μg) and 24 (24 SV) and 72 (72 SV) h starvation on food intake in *C. auratus* at 2 h ($n = 10$/group). (c) Food intake (mg) in *C. auratus* at 2 h postinjection. F + SAL: fish daily fed and injected with 1 μl saline; 24 SV + SAL: goldfish injected with 1 μl saline after 24 h starvation; 24 SV + neuropeptide Y-(27–36): goldfish injected with 5 μg neuropeptide Y receptor antagonist [D-Tyr$^{27-36}$, D-Thr^{32}] neuropeptide Y-(27–36) after 24 h starvation. Data are expressed as mean + S.E.M. $^{**}P < 0.01$ compared to control groups (F and F + SAL).

4. Discussion

Our results show for the first time that the i.c.v. administration of neuropeptide Y (1 μg) increases food intake at short time in goldfish. These results confirm previous reports where neuropeptide Y stimulates feeding in different groups of vertebrates: mammals such as rats (Clark et al., 1984; Levine and Morley, 1984; Stanley and Lei-

bowitz, 1984, 1985; Páez et al., 1991; Jewett et al., 1992), mouse (Morley et al., 1987) rabbit (Pau et al., 1988), squirrel (Boswell et al., 1993), pig (Parrot et al., 1986) and sheep (Miner et al., 1989); birds (Kuenzel et al., 1987; Richardson et al., 1995), and reptiles (Morris and Crews, 1990). Although commercial neuropeptide Y is only available from mammalian species (rat, porcine, sheep and human), our results show a stimulatory central action of neuropeptide Y (from mammalian origin) on food intake in goldfish. In this sense, and taking in mind the high conservation of neuropeptide Y sequence throughout phylogeny (Larhammar et al., 1993; Larhammar, 1996), the feeding regulation by neuropeptide Y-like factors could be one example of a highly conserved physiological process in vertebrate evolution.

The fact that the feeding increase by neuropeptide Y was observed during the first 2 h after the injection, but not during the next discrete interval (2–8 h), suggests that this polypeptide acts at short time in goldfish. A similar short time action of neuropeptides on feeding regulation in goldfish has been reported. Corticotropin-releasing factor, CRF, (de Pedro et al., 1993), β-endorphin (de Pedro et al., 1995a) and galanin (de Pedro et al., 1995b) modify food intake at 2 h postinjection, but not during the next 6 h. Bombesin and cholecystokinin alter ingestive behavior since 30 min after the treatment (Himick and Peter, 1994a,b). The neuropeptide Y-induced feeding has been also observed at very short time (30 or 60 min postinjection) in homeotherm (Levine and Morley, 1984; Stanley and Leibowitz, 1984, 1985; Pau et al., 1988; Páez et al., 1991; Richardson et al., 1995). Such early increase of food intake by neuropeptide Y can be conserved for some hours later, being reflected in all subsequent data of cumulative food intake. A certain stimulatory effect of neuropeptide Y (1 μg) on cumulative food intake at 8 h postinjection was observed in goldfish, although this increase was not statistically significant. In sight of these results, the augmentation of food intake at 8 h by neuropeptide Y in goldfish would reflect the stimulatory action of this peptide at short time (2 h).

The lack of effect of the neuropeptide Y i.p. administration on feeding discards a possible peripheral role and supports a central effect of this peptide in the regulation of ingestive behavior in fish. Similar results have been previously reported in mammals, where neuropeptide Y regulates feeding when is given centrally, but not effect has been described for this neuropeptide peripherally administered (Allen and Bloom, 1986; Leibowitz, 1995; Balasubramaniam, 1997; White and Martin, 1997). Nevertheless, our results do not preclude the hypothesis that NPY may be more rapidly metabolized in the periphery than in cerebrospinal fluid, as it has been reported for other neuropeptides (galanin: Harling and Holst, 1992; Tyr-MIF-1, Kastin et al., 1994). In fact, Stenfors et al. (1997) have demonstrated that the inactivation pathway of NPY in the brain is different from that found in the periphery. Other questions can not be ruled out, such as that the dosages used were inappropriate to exert a peripheral role in food intake regulation.

The use of antagonists has been helpful in understanding of neuropeptide effects on food intake. Particularly, to investigate the specificity of the stimulatory effect of neuropeptide Y on food intake in goldfish, we selected a general antagonist of neuropeptide Y receptors, [D-Tyr^{27,36}, D-Thr^{32}] neuropeptide Y-(27–36), which blocks the central neuropeptide Y-induced feeding increase in mammals (Balasubramaniam, 1997; Beck-Sickinger, 1997; Rabinivich et al., 1997). We have found that this neuropeptide Y receptor antagonist counteracted the neuropeptide Y-induced feeding behavior in goldfish, when it is i.c.v. administered before neuropeptide Y injection. This result suggests that the stimulatory effect of neuropeptide Y in goldfish is mediated through specific receptors, in accordance with data obtained in rat, where this same neuropeptide Y receptor antagonist reversed the feeding stimulation induced by neuropeptide Y (Myers et al., 1995). Nevertheless, neuropeptide Y-(27–36) is a general antagonist that can bind to different subtypes of neuropeptide Y receptors. Experiments to determine which neuropeptide Y receptor subtypes are involved in neuropeptide Y-induced feeding in goldfish are in course in our laboratory.

Moreover, we have found that neuropeptide Y-(27–36) does not modify by itself food intake, suggesting, as in other studies (de Pedro et al., 1995b), that the antagonist does not produce nonspecific behavioral effects, which would complicate the interpretation of the ability of neuropeptide Y-(27–36) to antagonize the neuropeptide Y-induced feeding. Such ability was likely not due to the production of malaise, since none of the fish treated with the antagonist showed retreat to the bottom of the aquaria, lowering of the dorsal fins and/or decreased locomotor activity, which are characteristic behavioral traits of malaise in goldfish (Himick and Peter, 1994b).

It is known that brain levels of neuropeptide Y can be altered by acute and/or chronic modifications in nutrient composition and ration of food intake in mammals (Beck et al., 1990, 1992; Leibowitz, 1995), showing that fasting increases hypothalamic content of this peptide (Yoshihara et al., 1996). In this sense, it has been suggested that neuropeptide Y can be involved in the feeding stimulation induced by food deprivation. In fact, several studies have confirmed this hypothesis in rat (Schwartz et al., 1995). Our results also support that neuropeptide Y could mediate the eating stimulated by fasting in goldfish. This is based on, first, food deprivation increases food intake, and this increase is similar the rise produced by i.c.v. administration of neuropeptide Y, and second, the neuropeptide Y receptor antagonist counteracted the feeding stimulation evoked by fasting. These results are consistent with previous reports in rat, where immunoneutralization of endogenous neuropeptide Y in paraventricular nucleus attenuates the hyperphagic response to starvation (Shibasaki et al.,

1993). Taking into account all findings together, we suggest that fasting would increase hypothalamic content of neuropeptide Y, which would be responsible of the higher food consumption. This possibility is supported by data obtained in salmonid fish, where neuropeptide Y-like expression increases in hypothalamus from food-deprived fish (Silverstein et al., 1998). Nevertheless, we can not rule out that another peptides and/or monoamines are involved in the feeding stimulation as response to starvation. In fact, it has been described that fasting induced changes in some central peptides and monoamines in mammals (Morley, 1987) and fish (de Pedro et al., 1998). Moreover, this could explain the fact that the neuropeptide Y receptor antagonist did not totally block the food intake increase by fasting in goldfish, observing a 50% reversion, similar value to obtained in rat (Schaffhauser et al., 1997).

In summary, the present study indicates that neuropeptide Y increases food intake in goldfish at central level, and this action of neuropeptide Y is mediated by specific neuropeptide Y receptors. In addition, our results provide evidence for a role of neuropeptide Y in the eating evoked by food deprivation in *C. auratus*.

Acknowledgements

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