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Alterations in Food Intake and Thyroid Tissue Content by Corticotropin-Releasing Factor in *Tinca tinca*

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The present experiments test the effects of intracerebroventricular injections of ovine corticotropin-releasing factor (1 µg) on food intake, plasma glucose levels and thyroid function at 8 h postinjection in tench. Food intake and thyroid triiodothyronine (T_3) content were significantly decreased after CRF treatment. Thyroid thyroxine (T_4) content and plasma glucose levels were not modified by this neuropeptide. The present results suggest that CRF plays a role on food intake regulation and thyroid gland activity in tench.

Key words: CRF, Thyroid hormones, Food intake, Teleosts.

Neuropeptides have recently been involved in the modulation of feeding behavior in fish. Suppressive actions on food intake by CRF (6), bombesin (16) and cholecystokinin (17) have been described in goldfish, as well as stimulatory effects of ß-endorphin in the same species (8).

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CRF, a 41-amino acid peptide, has been highly conserved throughout the evolutionary process (22) and it has been shown to be the most important physiological regulator in the mammalian secretion of ACTH, ß-endorphin and other proopiomelanocortin (POMC) derived peptides *in vivo* and *in vitro* (22). In addition, growing evidences suggest that CRF also acts in mammals as a neurotransmitter in the central nervous system integrating endocrine, behavioral and other physiological responses to stress (22). In teleost

fish, ovine corticotropin-releasing factor (oCRF) stimulates ACTH release *in vitro* from the rostral pars distalis of *Carassius auratus* (11) and *Oncorhynchus mykiss* (2) and stimulates POMC-derived peptides from goldfish neurointermediate lobe cells (24).

The CRF role in the control of appetite behavior in mammals has been extensively investigated for the last years. Feeding is inhibited after intracerebroventricular (ICV) CRF administration (9, 13) and, as it occurs in other CRF-induced behaviors, the decrease in food intake can be reversed by the CRF antagonist (18).

On the other hand, a number of recent studies in amphibians and birds have suggested that CRF is able to stimulate TSH secretion by the pituitary gland, and consequently, to activate the thyroid gland (5, 12, 20). Moreover, central oCRF injections not only stimulated thyroid gland activity, but also inhibited food intake and growth in *Rana perezi* tadpoles (4). Relationships between thyroid function and feeding have also been reported in fish (10, 14).

The aim of the present study was to investigate if central administration of CRF affects food intake and thyroid gland activity in one of the most interesting cyprinid species *(Tinca tinca)* for aquaculture purposes in Spain.

Materials and Methods

Animals.– Experiments were performed in tench, *Tinca tinca* (1.3 ± 0.44 g b. w.), provided by the "Centro Nacional de Ciprinicultura, Vegas del Guadiana", Badajoz, Spain. Fish were maintained during fall and winter under laboratory conditions (natural photoperiod and 19 ± 2 °C water temperature) in glass aquaria (50 l) with flowing and aerated tap water, and they were fed Sera Biogran food at a daily ratio of 1.6 % b. w. at 11:00 h. Animals were acclimated to a 12L:12D photoperiod (light on at 4:00 h am) and 20 ± 1 °C water temperature for two weeks prior to use.

Experimental procedure.– Ovine CRF (Sigma) was prepared and intracerebroventricularly injected as previously described (6). After a 24-hour food deprivation, animals were anesthetized in water containing tricaine methanesulphonate (MS-222, 1/10000), and fish were divided in two groups: a) 1 µl teleost saline (n = 24, control group); b) 1 µg oCRF dissolved in 1 µl saline (n = 24, CRF group).

Fish recovered equilibrium and usual swimming activity in anesthetic-free water within 1-3 min after injection. Subsequently, tench were transferred to 5 l aquaria and received preweighted food in excess (10 % b.w.). Due to the small size of tench, it was necessary to pool four animals per aquaria, thus the *n* was 6 for food intake and plasma glucose levels determination, and 24 for thyroid hormones.

Feeding quantification.– Food intake (F I) was measured at 8 h postinjection (minimum time needed for significant food intake evaluations in 4 fish pools), and computed as follows:

$$F\,I = W_i - (W_f \times F)$$

W_i = initial dry food weight; W_f = remaining dry food weight; and F = correction factor. It was calculated to evaluate the effect of water dissolution on food pellets during the feeding time. F represents the reduction in food weight after 8 h of immersion (F = 1.4) in the aquaria.

Plasma glucose determination.– Blood samples were collected by caudal section using heparinized capillary tubes in anesthetized fish at 8 h after injection. Plasma glucose levels were determined by the glucose-oxidase method (Glucose Trinder, Knickerbocker Labs.).

Thyroid hormones determination.– The lower jaws containing thyroid tissue were removed immediately, were frozen on dry ice and stored at –25 °C until assayed for thyroid hormones. Lower jaws were homogenized in methanol (3 ml/100 mg w. w.) and centrifuged for 15 min at 4500 rpm. The supernatant was collected, evaporated to dryness and reconstituted in 300 µl of RIA buffer. The thyroid hormones thus extracted represent the free thyroid T_4 and T_3 contents. The pellet was air-dried and subjected to overnight proteolytic digestion with pronase 5.8 % (Sigma) in Tris-HCl buffer at 37 °C. Reaction was stopped with methanol (3 ml) and, after centrifugation at 5000 rpm for 15 min, the supernatant was processed as it has been described above. This fraction represents bound-thyroglobulin T_4 and T_3.

T_4 and T_3 concentrations were determined by the highly sensitive and specific RIAs described by OBREGÓN *et al.* (21). The limits of detection were 2.5 pg and 0.7 pg/tube for T_4 and T_3, respectively. Intra- and interassay coefficients of variation were 4.62 and 16.5 for T_3 and 3.36 and 7.78 for T_4.

All data were expressed as mean ± SEM. Statistical differences were determined by a Student's *t* test.

Results

The effect of intracerebroventricular oCRF administration on feeding behavior in tench is presented in fig. 1. A significant reduction of food intake in CRF-injected compared to vehicle-injected tench was found. There were no significant differences in plasma glucose levels after CRF treatment (control: 2.65 ± 0.56 mg/ml; CRF: 2.07 ± 0.17 mg/ml).

Figure 2 shows free and bound fractions of thyroid hormone after CRF treatment in tench. CRF induced a significant reduction in both, free and bound fractions of thyroid T_3. There were no significant changes, however, either in thyroid T_4 content or in T_3/T_4 relation after treatment with this neuropeptide.

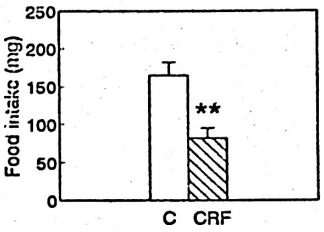

Figure 1. *Food intake after intracerebroventricular CRF treatment at 8 h postinjection in* Tinca tinca. Values are means ± SEM; n = 6/group. ** $p < 0.005$.

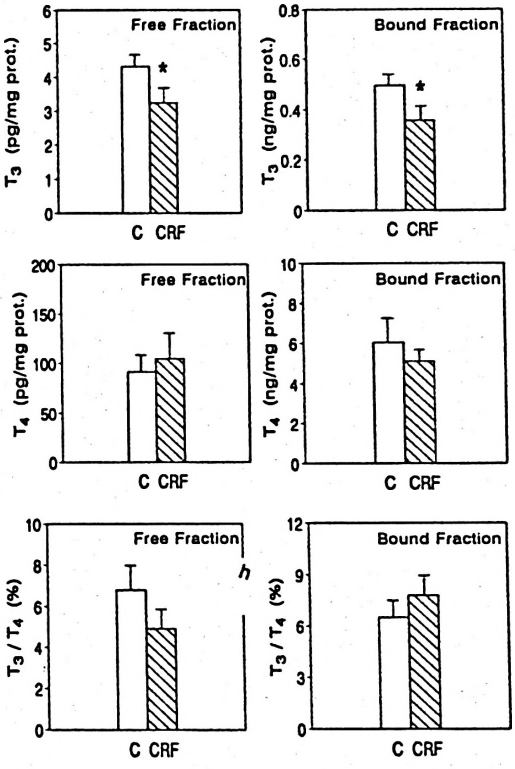

Figure 2. *Thyroid content of free (A) and bound (B) fractions T_3 and T_4, and the ratio T_3/T_4 at 8 h postinjection in tench.*
Values are means ± SEM; n = 24/group. * $p < 0.05$.

Discussion

The present results show that CRF is involved in the central feeding regulation in tench, acting as a potent anorexic neuropeptide. Similar results have also been described in mammals (13, 18) and, in poikilotherm species recently in our laboratory, particularly in frog tadpoles (4) and goldfish (6). In view of these results, it appears that CRF or a CRF-like peptide plays a role in the central feeding regulation in vertebrates.

The lack of CRF effect on plasma glucose levels is in agreement with previously reported data in rats (1). A hypothetical glucose reduction induced by food intake inhibition after CRF treatment, could be counteracted by the stressful effect of this peptide increasing glucose titers (3), explaining the lack of CRF effect on plasma glucose levels in tench. However, recent experiments at our laboratory indicate that refeeding after food deprivation, but not CRF administration, is the signal that triggers the increase of plasma glucose levels in goldfish (7). In this case, the CRF role as a blood sugar regulator would not be so relevant.

Starvation in fish has been reported to depress plasma T_3 levels, but not T_4 (15). In this sense, both the feeding inhibition and the thyroid T_3 reduction after CRF treatment in tench could be associated. The hypothesis that T_3 reduction in CRF-treated fish may be a consequence of food intake reduction is corroborated by the fact that a shortage or complete lack of food suppresses thyroid hormone release from the thyroid in some vertebrates (10, 15).

The present results suggest that CRF action on thyroid activity in tench mainly affects T_3 synthesis and secretion, since T_4 contents and T_3/T_4 ratios remain unchanged. A direct activation of the pituitary-thyroid axis by CRF administration has been reported (5, 12, 20). In prometamorphic tadpoles acute treatment with CRF induces a significant increase in whole body concentration of T_4 without T_3 changes (12). By contrast, in the present study a stimulatory CRF effect on thyroid activity cannot be assumed, since other thyroid activity parameters, such as plasma titers were not assessed due to the small size of tench which does not allow the obtention of enough blood volumes to determine plasma thyroid hormones. Thus, a CRF-stimulated T_3 release into circulation cannot be discarded, a possibility that may explain the final decrease in thyroidal T_3 content. On the other hand, ACTH and/or corticosteroids in some teleosts have been shown to depress pituitary-thyroid axis (19, 23). Thus, the inhibition of thyroid function by CRF in tench can be mediated by either food intake reduction and/or pituitary/adrenal axis stimulation, which could overlap the hypothetical stimulatory action of the neuropeptide on thyroid. An *in vitro* experimental approach could elucidate this interesting question.

The present study supports a central role of CRF on food intake regulation in tench and suggests a relationship between CRF and pituitary-thyroid axis in teleost.

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N. DE PEDRO, B. GANCEDO, A. L. ALONSO-GÓMEZ, M. J. DELGADO y M. ALONSO-BEDATE. *Alteraciones en la ingesta y el contenido tiroideo por el factor liberador*

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Se estudian en tenca *(Tinca tinca)* los efectos de la administración intracerebroventricular de 1 µg del factor liberador de corticotropina ovino (CRF) sobre la ingesta, los niveles plasmáticos de glucosa y la función tiroidea, a las 8 h del tratamiento. La ingesta y el contenido tiroideo de T_3 se reducen significativamente después del tratamiento, sin que se modifique el contenido tiroideo de T_4 ni los niveles plasmáticos de glucosa. Los resultados sugieren que el CRF desempeña un papel en la regulación de la ingesa y en la actividad del tiroides en las tencas.

Palabras clave: CRF, Hormonas tiroideas, Ingesta, Teleósteos.

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