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CRF Effect on Thyroid Function Is Not Mediated by Feeding Behavior in Goldfish

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DE PEDRO, N., B. GANCEDO, A. L. ALONSO-GOMEZ, M. J. DELGADO AND M. ALONSO-BEDATE. *CRF effect on thyroid function is not mediated by feeding behavior in goldfish.* PHARMACOL BIOCHEM BEHAV **51**(4) 885-890, 1995.—In the present study we examined the effects of acute corticotropin-releasing factor (CRF) administration and refeeding treatment on glucose levels and thyroid hormones (plasma levels and thyroid contents) in 48-h food-deprived goldfish. Central CRF administration (2 μg) decreased food intake and the thyroid T_3 free fraction, without significantly modifying either thyroid hormones bound fractions (T_3 and T_4) or plasma glucose levels. Subsequently, we tested whether CRF affects thyroid activity by itself, or whether this effect is mediated by CRF-induced feeding reduction. CRF treatment in fasted fish reduced thyroid-free T_3 and increased thyroid-free T_4, which could be mediated by a decreased intrathyroidal 5′-monodeiodinase activity. These data suggest a CRF effect upon thyroid activity independent of feeding reduction. On the other hand, refeeding after 48-h fasting caused a significant increase in thyroid free T_4 content and plasma thyroid hormone levels. Thus, a relationship between nutritional status and thyroid function, which could overlap with CRF effects, cannot be discarded. Plasma glucose levels were only significantly modified by refeeding, which seems to be the signal triggering the increase in glucose titers. Our results support the existence of both CRF-thyroid activity and nutritional status-thyroid function interactions in goldfish.

Goldfish CRF Fasting Glucose Thyroid hormones Thyroid *Carassius* T_3 T_4 Teleosts

RECENTLY, a wide range of neuroendocrine, physiologic, and behavioral effects after corticotropin-releasing factor (CRF) administration has been described (10). Particularly, CRF has been demonstrated to be a potent anorexic agent after central administration in mammals (15,25). Recent studies carried out in our laboratory have also shown an inhibitory effect of CRF on food intake in nonmammalian vertebrates, tadpoles (5), and goldfish (6).

CRF seems to participate in the regulation of the hypothalamic-pituitary-thyroid (HPT) axis. It has been shown a thyrotropic activity of CRF in chick embryos (22), tadpoles (9, 14), and adult amphibians (8,19). This CRF effect may be mediated and/or intensified by a concomitant increase in corticosteroids, which are known to increase hepatic 5′-monodeiodinase activity and plasma levels of 3,5,3′-triiodo-L-thyronine (T_3) in chick embryos (7). However, it seems that glucocorticoid effects on thyroid axis can vary depending on the age of the animal and/or the species, because ACTH and corticosteroids decrease blood thyroid hormone levels in adult domestic fowl (24) and in several teleost fish (19,21).

On the other hand, studies in birds (20), mammals, and teleost fish indicate that thyroid function is altered by acute and/or chronic modifications in nutrient composition and ration (11). In particularly, long- and short-term starvation decreases plasma levels of both, L-thyroxine (T_4) and T_3 in salmonids (12,16).

In the present work we studied the effects of CRF on plasma glucose levels and thyroid T_3 and T_4 contents in goldfish. Taking into account these relationships (i.e., CRF-food intake and nutritional status-thyroid hormones), we separately examined the effects of feeding and CRF treatment on thyroid activity. This approach allowed us to determine whether thyroid hormone modifications are a consequence of food intake variations after CRF treatment or a direct effect of CRF on the thyroid axis.

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METHOD

Animals

Experiments were carried out in goldfish, *Carassius auratus*, (8–20 g body wt. and 6–7 cm standard length) obtained from a commercial supplier in Madrid.

Fish were maintained at the laboratory in glass aquariums (50–100 l) in flowing and aerated tapwater, under a natural photoperiod and 20 ± 1°C water temperature. All fish were fed Sera Biogran pellets at a daily ration of 0.8–1% body wt. at 1100 h. Animals were acclimated to these conditions in spring (12 L : 12 D, Experiment 1) and winter (10 L : 14 D, Experiment 2) for at least 2 weeks before experimental use.

Experimental Procedure

After 48-h food deprivation, animals were anaesthetized with tricaine methanesulphonate (MS-222; 1 : 10,000). Immediately after the fish lost equilibrium, we determined their length and body weight and intracerebroventricularly (ICV) injected them. All injections were performed between 1030 and 1130 h. The CRF dose and procedure used in these experiments were based on previous studies (6). Briefly, ICV injections were performed free-hand through the central junction between the parietal and frontal bones. The accuracy of injection placement into the ventricular region of the fish brain was established in preliminary experiments (6).

Experiment 1.

Effect of CRF on food intake, thyroid T_3 and T_4 contents, and plasma glucose levels. Fourteen goldfish were divided in the following two groups: a) saline + fed group (n = 7): 1 μl teleost saline (20 mg Na_2CO_3 per 100 ml of 0.6% NaCl); and b) CRF + fed group (n = 7): 2 μg ovine CRF (oCRF) dissolved in 1 μl teleost saline. Each goldfish was transferred into its own 5-l aquarium and recovered equilibrium and swimming activity in anaesthetic-free water within 1–3 min after the injection. Subsequently, all fish received preweighed food in excess (8–10% body wt.).

Feeding quantification. Food intake (FI) was measured at 2 h postinjection and computed as in previous studies (6): FI = $W_i - (W_f \times F)$, where W_i = initial dry food weight, and W_f = remaining food weight, after pellets were dried.

To correct the effect of water dissolution on food pellets during the feeding time, a correction factor, F, was calculated. F represents the reduction in food weight after 2 h of immersion (F = 1.09) in the aquarium.

Experiment 2.

Differential effects of feeding alteration and CRF treatment on thyroid T_3 and T_4 contents and plasma thyroid hormones and glucose levels. Thirty-three goldfish were divided in the following three groups: a) saline + fasted group (n = 11): 1 μl saline; b) CRF + fasted group (n = 11): 2 μg oCRF in 1 μl saline; and c) saline + fed group (n = 11): 1 μl saline. Each goldfish was also transferred into its own 5-l aquarium and recovered equilibrium and swimming activity in anaesthetic-free water within 1–3 min after the injection. Subsequently, the fed group received preweighed food in excess (8–10% body wt.), whereas food deprivation continued for 8 h after either saline or CRF administration in fasted groups.

Plasma glucose and thyroid hormones were determined in both Experiments 1 and 2 as follows:

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

Thyroid hormone determination. The lower jaws containing the thyroid tissue (27) were removed, frozen on dry ice, and stored at −25°C until assayed for thyroid hormones. For extraction, lower jaws were homogenized in methanol (12 ml/g wet weight) and centrifuged for 15 min at 4000 rpm. The supernatant was collected, evaporated to dryness, and reconstituted in 0.5 ml of radioimmunoassay buffer. The thyroid hormones thus extracted constituted the free thyroid T_4 and T_3 content. The pellet was air-dried and subjected to overnight proteolytic digestion with pronase 0.58% (Sigma, St. Louis, MO) in Tris-HCl buffer at 37°C. Reaction was stopped with

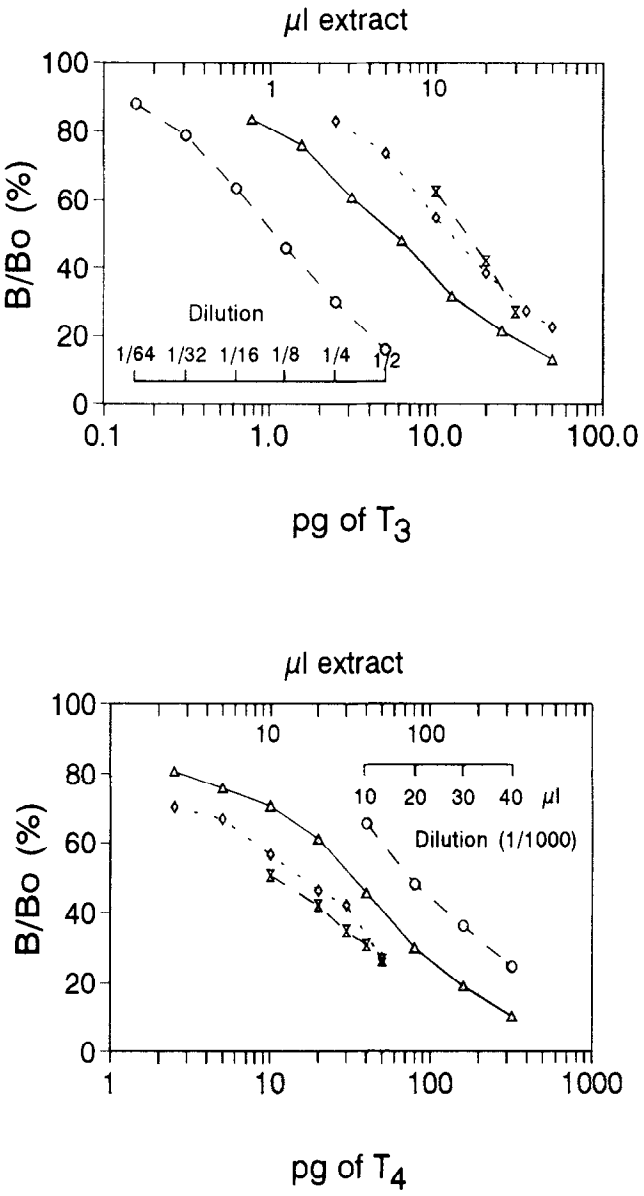

FIG. 1. Comparison of T_3 and T_4 standard curves (▲–▲–▲) with serial dilutions of bound fraction (●–●–●), or different volumes of free fraction (×–×–×) and plasma (♦–♦–♦) samples. Each point represents the average of duplicate determinations for samples. Standard curves represent the average of three different standard curves.

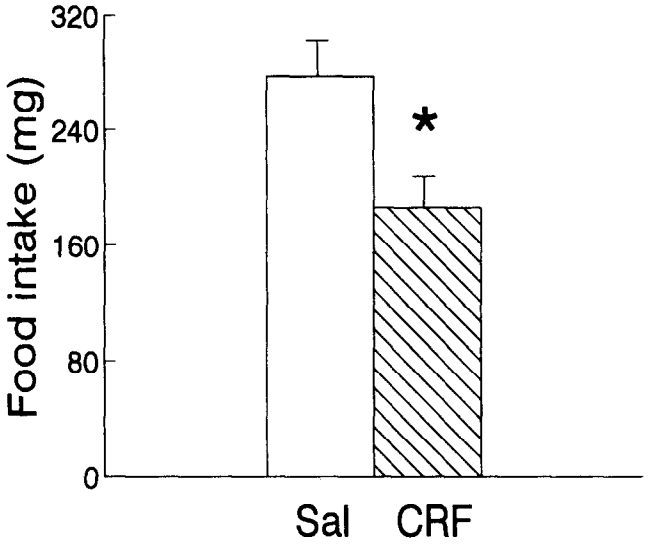

FIG. 2. Food intake after intracerebroventricular CRF treatment at 2 h postinjection in 48-h food-deprived goldfish. Data are expressed as mean ± SEM. *$p < 0.01$ compared to the saline group. Saline, $n = 7$ (open column); CRF-treated, $n = 6$ (striped column).

methanol (3 ml) and, after centrifugation at 4000 rpm for 15 min, the supernatant was processed as described earlier and reconstituted in 1 ml of RIA buffer. This fraction represents bound-thyroglobulin T_4 and T_3.

Plasma thyroid hormones were extracted and measured as previously described by Morreale et al. (26). Briefly, iodothyronines were back-extracted into an aqueous phase from chloroform-methanol extracts (2 : 1) with 0.05% $CaCl_2$, followed by further purification with Bio-Rad AG 1X2 resin columns. The final elutes were evaporated to dryness and taken up in 0.3 ml of RIA buffer. Results were calculated considering individual recovery data obtained by the addition of tracer amounts of (131I)T_4 and (^{125}I)T_3 to each sample. The possible interference of these tracers in the RIAs was not significant. Recoveries of extracted iodothyronines were 68.4 ± 6.7% for (^{125}I)T_3 and 64.78 ± 7.1% for (^{131}I)T_4.

T_4 and T_3 were determined by highly sensitive and specific RIAs described by Obregón et al. (28). The limits of detection were 2.5 and 0.78 pg/tube for T_4 and T_3, respectively. The intra- and interassay coefficients of variation were 4.16 and 8.55% (dose 1.56 pg, $n = 9$), 9.05 and 18.26% (dose 25 pg, $n = 10$) for T_3; and 4.63 and 6.69% (dose 10 pg, $n = 9$), 7.91 and 14.85% (dose 160 pg, $n = 10$) for T_4.

Parallelism was determined by comparing the displacement curves obtained with serial dilutions or different volumes of either extracted plasma or thyroid samples with standard curves (T_3 and T_4). The data were logit-log transformed to allow the calculation of slopes by linear regression. Afterward, parallelism was statistically tested by one-way analysis of variance between the slopes of standards and sample dilutions.

All data are expressed as mean ± SEM. In each experiment Student's t-test was performed to detect statistical differences between control and experimental (CRF treatment, food intake alteration) groups.

A probability level of $p < 0.05$ was considered to be statistically significant.

TABLE 1

PLASMA GLUCOSE LEVELS IN 48-h FOOD-DEPRIVED (*Carassius auratus*) AT 8-h POSTINJECTION

Experiment	Treatments: Injection	+ Nutritional Status	n	Glucose (mg/ml)
1	Saline	Fed	7	2.46 ± 0.29
	CRF	Fed	7	2.68 ± 0.39
2	Saline	Fasted	5	0.72 ± 0.17
	CRF	Fasted	6	0.62 ± 0.22
	Saline	Fed	5	2.07 ± 0.47*

Data are expressed as mean ± SEM.
*$p < 0.025$, compared to saline + fasted group.

RESULTS

Serial dilutions of either plasma or thyroid tissue-extracted samples gave parallel displacements to the T_3- and T_4-standard curves (Fig. 1). Significant differences were not observed ($p > 0.05$) between the slopes of standards and sample dilutions.

The effect of ICV CRF administration on feeding behavior in goldfish (Experiment 1) is presented in Fig. 2. CRF caused

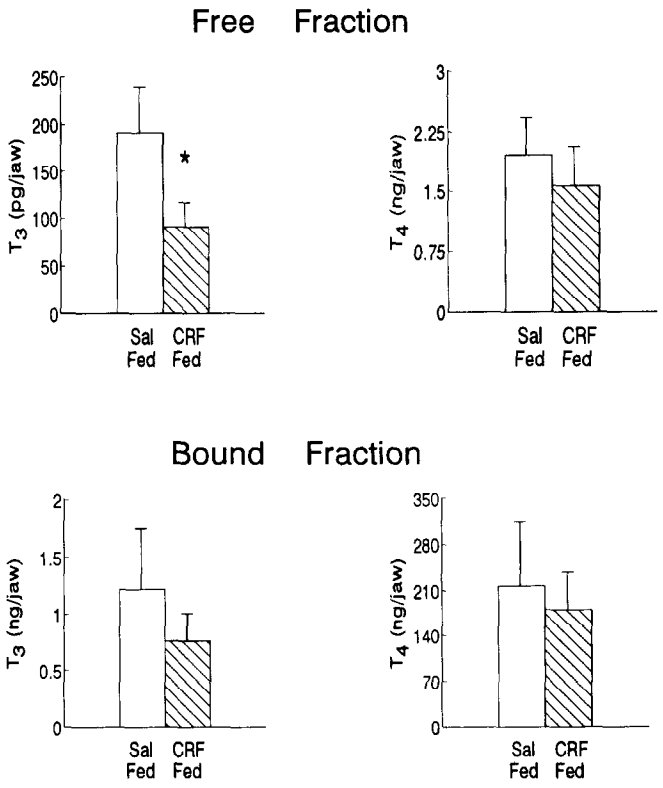

FIG. 3. Thyroid contents of T_3 and T_4 free fraction (upper panel) and T_3 and T_4 bound fraction (lower panel) at 8 h postinjection in 48-h food-deprived goldfish (Experiment 1). Data are expressed as mean ± SEM. *$p < 0.05$ compared to the saline group. Saline + Fed group, $n = 6$ (open column); CRF + Fed group, $n = 7$ (striped column).

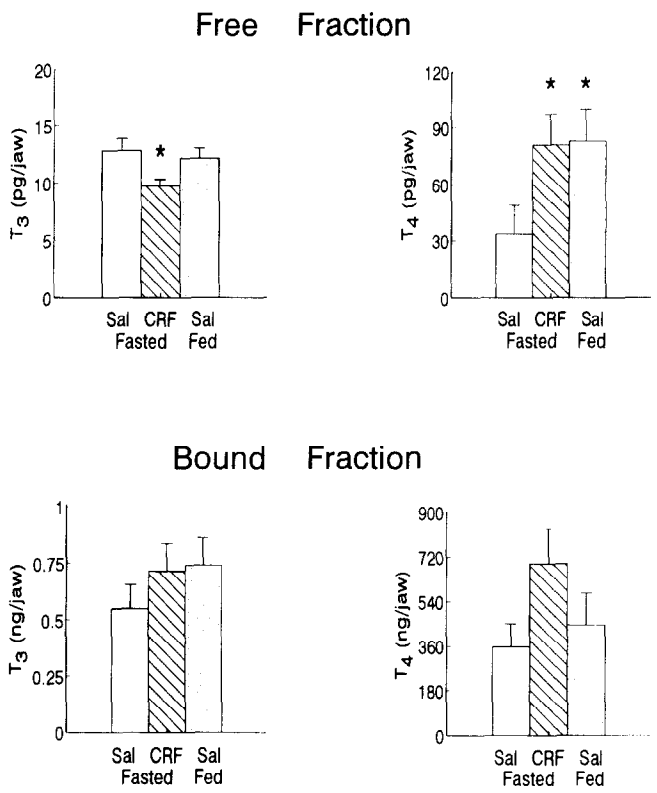

FIG. 4. Thyroid contents of T_3 and T_4 free fraction (upper panel) and T_3 and T_4 bound fraction (lower panel) at 8 h postinjection in 48-h food-deprived goldfish (Experiment 2). Data are expressed as mean ± SEM. *$p < 0.05$ compared to the saline + fasted group. Saline + Fasted group, $n = 9$ (open column); CRF + Fasted group, $n = 11$ (striped column); and Saline + Fed group, $n = 10$ (dotted column).

a significant ($p < 0.01$) reduction (33%) in food intake with respect to the saline group.

Table 1 shows that there were no significant differences in plasma glucose levels between saline and CRF fed-groups in Experiment 1. Similar plasma glucose levels were found in the saline + fed group (Experiment 2). By contrast, these values were significantly higher ($p < 0.025$) than those found in both fasted groups, independently of injection (saline or CRF, Experiment 2).

CRF treatment induced a significant reduction of thyroid T_3 free fraction ($p < 0.05$) (Fig. 3). However, as also shown in Fig. 3, there were not significant changes in either T_3 bound fraction or T_4 free and bound fractions.

In fasted goldfish the ICV administration of CRF also reduced T_3 free fraction ($p < 0.05$), but increased T_4 free fraction ($p < 0.05$), in relation to the saline + fasted group (Fig. 4). No significant differences in thyroid hormone bound fractions were observed after CRF treatment. A significant increase ($p < 0.05$) in T_4 free fraction was also observed in the saline + fed group compared to the saline + fasted group. Neither free T_3 nor T_3 and T_4 bound fractions were significantly modified by feeding.

Figure 5 shows plasma thyroid hormone levels in Experiment 2. Significant differences between saline and CRF-fasted fish were not observed. T_3 and T_4 levels were higher (about threefold) in the saline + fed group than in the saline + fasted one ($p < 0.01$ for T_3, and $p < 0.05$ for T_4).

DISCUSSION

The present results confirm previous reports that CRF is involved in the central regulation of feeding, acting as a potent anorexic neuropeptide in different vertebrates [mammals (15,25,29); amphibians (5); and fish (6)].

Plasma glucose levels are closely related to feeding in fish (13,31); thus, the feeding reduction induced by CRF would probably bring about an alteration in glucose titers. We have not found a modification in plasma glucose levels after CRF treatment as was reported in rats (2). This lack of CRF effect

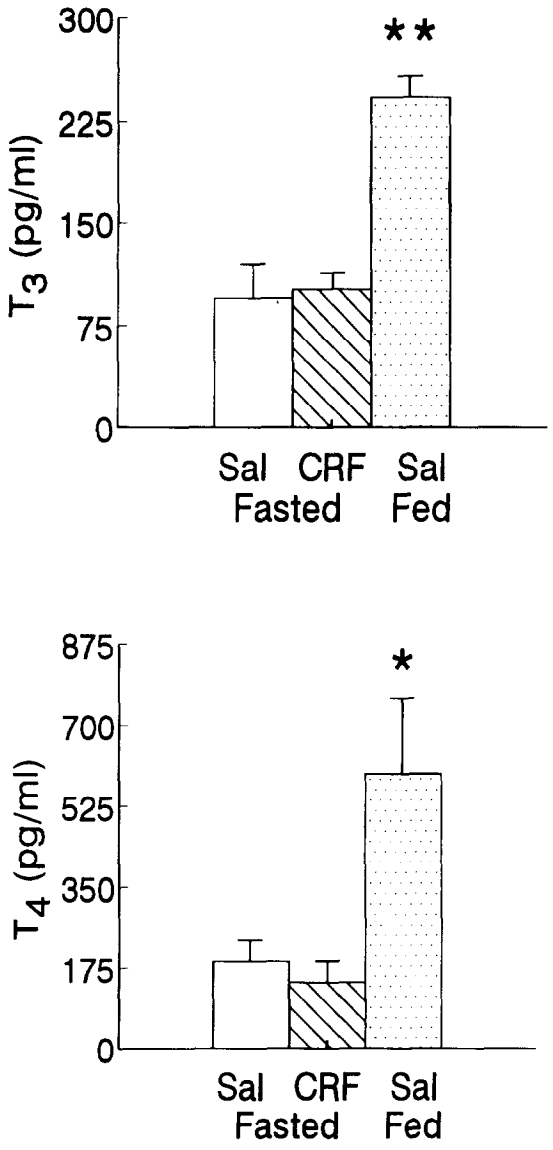

FIG. 5. Plasma thyroid hormone levels (mean ± SEM) at 8 h postinjection in 48-h food-deprived goldfish (Experiment 2). *$p < 0.05$; **$p < 0.01$ compared to the saline + fasted group. Saline + Fasted group, $n = 7$ (open column); CRF + Fasted group, $n = 8$ (striped column); and Saline + Fed group, $n = 6$ (dotted column).

on circulating glucose in goldfish could be interpreted to be an overlap between the glucose reduction induced by food intake inhibition after CRF treatment and the well-known stressful effect of this peptide-increasing glucose titers (4). The second experiment performed in our study indicates that CRF by itself does not modify plasma glucose levels compared to the saline-fasted group. However, the fact that glucose titers in fasted goldfish were significantly lower than in fed groups suggests that feeding behavior increases plasma glucose levels. These results indicate that refeeding triggers the increase in glucose titers, supporting previous reports in salmonid fish, where feeding quickly rises plasma glucose (31).

We found a concomitant reduction in food intake and thyroid T_3 free fraction in CRF-treated animals. Considering that food intake alters thyroid activity in fish (11), it could be possible that both responses were related, and in consequence T_3 reduction in CRF-treated goldfish may be due to the CRF-induced reduction in food intake. In this way, the results from Experiment 2 demonstrate that CRF is the only factor responsible for the thyroid free T_3 reduction, whereas refeeding does not modify thyroid T_3 content. To date, we cannot determine whether CRF acts directly on thyroid axis and/or whether this effect of CRF is mediated via ACTH and glucocorticoids.

The ICV CRF administration in 48-h food-deprived fish significantly increased thyroid T_4 free fraction. This increase in T_4 content associated with a T_3 reduction could be justified by a decrease in intrathyroidal 5'-monodeiodinase activity. This possibility is supported by recent studies suggesting that the intrathyroidal conversion of T_4 to T_3 or rT_3, and even their preferential secretion, can be modulated by feeding behavior (1) or thyrotropin-releasing hormone (TRH) and thyroid stimulating hormone (TSH) modifications (30).

The different thyroid hormone content (free fraction) observed in saline-fed groups (Experiment 1, Fig. 3; Experiment 2, Fig. 4) can be attributed to seasonal variations in thyroid activity (3).

CRF administration induced a significant increase of thyroid free T_4 in fasted goldfish, similar to that obtained in the refed fish. By contrast, we did not find statistical differences in T_4 free fraction after CRF treatment in the first experiment. This discrepancy could be due to a stimulatory effect of feeding on thyroid T_4 free fraction in goldfish, as was reported for plasma thyroid hormones in rainbow trout (12,16). Food intake could determine T_4 increases in saline with respect to CRF-treated fish (food intake reduced), which disguise the T_4 increase promoted by CRF. Nevertheless, we cannot rule out that this discrepancy could be due to a seasonal variation of thyroid activity as mentioned earlier.

The present finding that refeeding increases plasma thyroid hormone levels (T_3 and T_4) after 48-h food deprivation agrees with previous studies in salmonids. Flood and Eales (12) reported elevations in both plasma T_3 and T_4 4 h after refeeding in rainbow trout starved for either 3 or 6 days (16,17). In summary, the ingestion of a single meal following long- or short-term starvation increases plasma thyroid hormone levels in fish. This stimulatory action of refeeding on thyroid hormones has also been reported in other vertebrates, where a single meal reverted the suppressive effect of starvation on TRH and TSH actions (18,23).

Our data provide new evidence on the relationship between feeding behavior and thyroid hormones, showing for the first time that the CRF effect on thyroid hormones is not mediated by food intake reduction. Nevertheless, it is necessary to investigate whether CRF acts on thyroid activity either directly or via adrenal steroids.

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