α,-ADRENERGIC AND DOPAMINERGIC RECEPTORS ARE INVOLVED IN THE ANORETIC EFFECT OF CORTICOTROPIN-RELEASING FACTOR IN GOLDFISH

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Summary

This study investigates the noradrenergic and/or dopaminergic receptors subtypes involved in the anorectic action of CRF in goldfish. Agonists and antagonists of α,- and α,-adrenoceptors, and D,- and D,-dopaminergic receptors were intracerebroventricularly (ICV) administered alone or in combination with CRF in the case of antagonists. Food intake and hypothalamic content of catecholamines and their metabolites were measured at 2 h postinjection. On one hand, α,-adrenergic receptor antagonist, but not α,-, blocked the anorectic effect of CRF. Moreover, we found a blockade of CRF-induced anorectic action by pretreatment with specific D,- and D,-dopaminergic antagonists. On the other hand, the ICV administration of CRF reduced hypothalamic norepinephrine (NE) and dopamine (DA) content, without modifications in their metabolism. Thus, our results suggest that the anorectic effect of CRF appears to be mediated by α,-adrenergic and dopaminergic receptors in fish. Second, the reduction in hypothalamic NE and DA synthase could be due to a direct effect of CRF treatment and/or a secondary effect of food intake reduction.

Key Words: corticotropin releasing factor, food intake, goldfish, receptor antagonist, norepinephrine, dopamine, catecholamine

Corticotropin-releasing factor (CRF) is a 41-amino acid neuropeptide distributed widely in the central nervous system (CNS), and it has been involved in the regulation of several neuroendocrine, physiological and behavioral functions (1,2), such as the control of feeding behavior (3,4). The administration of CRF into the hypothalamus or into the brain ventricles induces a significant food intake decrease in all vertebrates so far studied: mammals, -rat (5,6), mouse (7), pig (8) and monkey (9,10)-, amphibians, -Rana perezi tadpoles (11)-, and fish, -goldfish (12) and tench (13)-. All these observations indicate that the anorectic function of CRF is highly conserved in vertebrates.

It is well established that the anorectic effect of CRF is independent of pituitary-adrenal activation in different mammalian species (3,6,8,14). Recent studies in goldfish have demonstrated cortisol increases after both intracerebroventricularly (ICV) and intraperitoneally (IP) CRF administration, but only ICV injections decrease food intake (15). The lack of feeding-depressant effect of peripheral CRF administration, together with the blockade of CRF-induced feeding reduction by the specific antagonist α-Helical CRF[84], clearly substantiates a central mediation of the anorectic effect of CRF, which is independent of pituitary-adrenal activation (15).

The hypothalamus is the main brain site involved in CRF-induced food intake reduction, particularly the paraventricular nucleus (PVN) (4). Several findings have indicated a possible interaction between CRF and the hypothalamic catecholaminergic system in vertebrate feeding regulation. Thus, direct
monoaminergic innervation of CRF neurons in the PVN has been evidenced in mammals, suggesting a modulation of hypothalamic CRF secretion by monoaminergic systems (2,16). Moreover, it has been shown changes in central neurotransmitter systems associated with CRF-induced appetitive changes in mammals (1,3). In this sense, studies carried out in our laboratory have shown a concomitant reduction in feeding and hypothalamic content of norepinephrine (NE) and dopamine (DA) in CRF-injected goldfish, being these effects reversed by the antagonist a-Helical CRF, (15). At least two hypothesis could explain these results. On one hand, considering the role played by catecholamines in feeding behavior regulation in mammals (4,17), it can be suggested that alterations in noradrenergic and/or dopaminergic systems could be acting as mediators of the CRF anorectic effect. Nevertheless, considering that feeding alters brain catecholamines content and their metabolism (18,19), it cannot be ruled out that the modification in NE and DA hypothalamic contents was not the consequence of the CRF-induced food intake reduction. To elucidate these questions, exploring if the noradrenergic and dopaminergic receptors are involved in the anorectic action of CRF, the present study has been performed. First, the effect of a,- and a,-adrenergic and D,- and D,-dopaminergic receptors agonists and antagonists (alone or in combination with CRF) on food intake in goldfish has been tested. Second, possible modifications in hypothalamic catecholaminergic system have been analyzed.

Methods

Animals

This study was performed on goldfish, Carassius auratus, (5.5±1.5 g body weight, bw) provided by a commercial supplier. Fish were acclimated to natural photoperiod and 21±2°C water temperature for 2 weeks before experiments were carried out. Feeding consisted in Sera Biogran pellets at a daily ration of 1% bw provided 3 h after the light onset.

Drugs

Ovine CRF, clonidine, phenylephrine, prazosin, sulpiride and yohimbine were purchased from Sigma (Spain) and quinpirole, R-SCH 23390 and R-SKF 38393 were from RBI (USA). Drugs were dissolved in teleost saline (20 mg Na,CO, /100 ml of 0.6% NaCl) alone or supplemented with glacial acetic acid (5%) for sulpiride, quinpirole and R-SKF 38393.

The CRF dose (1 µg) was chosen from the dose-response curves previously obtained in goldfish (12). Similarly, the doses of agonists (1 µg) and antagonists (10 µg) were chosen on the basis of the reported doses in previous studies (20).

Experimental procedure

Intracerebroventricular injections were accomplished with a 0.3 mm Microlance needle connected to a 5 µl Hamilton microsyringe with an 10-Venocath cannula in anesthetized fish (tricaine metanesulfonate, MS-222, 1:10,000). Detailed procedure of ICV injection has been published elsewhere (12,13). Fish recovered normal swimming activity in 1-2 min and received preweighed food (7% bw) in individual aquaria. According to the model of food deprivation-induced feeding, goldfish were food-deprived the day before the experiment, and the effect of treatments on food intake was determined 2 h after injections. The feeding quantification procedure has been previously reported (12,13).

Experiment 1. a-Adrenergic receptors as mediators of the CRF-induced anorectic effect in goldfish. Seventy two goldfish were divided into eight groups which received two sequential (separated by 10 min) ICV injections (1 µl each) as follows: (1) 2 injections of 1 µl saline; (2) saline injection followed by the a1 agonist phenylephrine (10 µg) injection; (3) saline injection followed by the a2 agonist clonidine (10 µg) injection; (4) saline injection followed by CRF (1 µg) injection; (5) saline injection followed by the a1 antagonest prazosin (10 µg) injection; (6) prazosin (10 µg) injection followed by CRF (1 µg) injection; (7) saline injection followed by the a2 antagonist yohimbine (10 µg) injection; (8) yohimbine (10 µg) injection followed by CRF (1 µg) injection. Hypothalamus were collected at 2 h after ICV injections and stored frozen (-80°C) until catecholamines determination.

Experiment 2. Dopaminergic receptors as mediators of the CRF-induced anorectic effect in goldfish. Eight groups (n=9/group) of fish received two consecutive (separated by 10 min) ICV injections (1 µl each): (1) 2 injections of 1 µl saline; (2) saline injection followed by the D, agonist SKF 38393 (10 µg) injection; (3) saline injection followed by the D, agonist quinpirole (10 µg)
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injection; (4) saline injection followed by CRF (1 μg) injection; (5) saline injection followed by the D₁ antagonist SCH 23390 (10 μg) injection; (6) SCH 23390 (10 μg) injection followed by CRF (1 μg) injection; (7) saline injection followed by the D₂ antagonist sulpiride (10 μg) injection; (8) sulpiride (10 μg) injection followed by CRF (1 μg) injection. Hypothalamus were collected and stored as above described.

Catecholamines determination
Norepinephrine (NE), epinephrine (E), 3-methoxy-4-hydroxyphenylglycol (MHPG), dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC) were quantified by high-performance liquid chromatography (HPLC) with coulometric detection, as previously described (15). Briefly, hypothalami were sonicated in 120 μl 0.2 N perchloric acid (PCA) containing 0.4 mM sodium bisulfite, 0.4 mM EDTA and 25 ng/ml of 3,4-dihydroxybenzilamine (DHBA) as internal standard. After centrifugation the supernatant was filtered and 25 μl was injected into the HPLC/EC system. The analytical column was C18, 125x4.6 mm internal diameter, 5 μm particle size. The potentials of the electrodes were +350 mV for conditioning cell, and +50, -250 mV for the analytical cells. The mobile phase (flow rate=1 ml/min) consisted of 10 mM citric acid, 5 mM disodium phosphate, 0.05 mM EDTA, 0.12 mM sodium octanesulfonate and 3% methanol (pH 3). The amount of catecholamines in the samples were calculated as the area under peaks, and expressed as nanograms per hypothalamus.

Statistics
Statistical significance of the data was determined by one-way analysis of variance (ANOVA) and Duncan's multiple range test for multigroup comparisons. A probability level of p<0.05 was considered statistically significant.

Results
Figure 1 shows the effect of ICV administration of α₁- and α₂-adrenergic agonists and antagonists alone or in combination with CRF on feeding in goldfish. Food intake was not significantly modified by either α₁ or α₂ agonists. The α₁ antagonist, prazosin, antagonized the inhibitory effect of CRF on feeding. The α₂ antagonist, yohimbine, by itself reduced food intake, being this reduction additive to the CRF-evoked inhibition.

Food intake (mg) after ICV administration of 1 μl saline; 10 μg phenylephrine; 10 μg clonidine; 1 μg CRF; 10 μg prazosin; both 10 μg prazosin + 1 μg CRF; 10 μg yohimbine; both 10 μg yohimbine + 1 μg CRF at 2 h postinjection in goldfish (Carassius auratus). Data are expressed as means±SEM, (n=9/group). * p<0.05, ** p<0.01 compared to saline group.

Fig. 1
The hypothalamic content of NE, its metabolite MHPG and the MHPG/NE ratio after ICV administration of α-adrenergic agonists and antagonists alone or combined with CRF are shown in Figure 2. Norepinephrine content decreased significantly in CRF-injected fish with respect to controls. This reduction was blocked by both antagonists, α1 and α2, but only yohimbine counteracted significantly the CRF-induced NA reduction. The hypothalamic content of MHPG was the highest in fish injected with both CRF and yohimbine, but the ratio MHPG/NE was not modified by the different treatments.

**Fig. 2**

Hypothalamic content of a) norepinephrine, b) MHPG and c) the MHPG/NE ratio after ICV administration of α-adrenergic agonists and antagonists alone or combined with CRF at 2 h postinjection in goldfish (*Carassius auratus*). 1 μl saline; 10 μg phenylephrine; 10 μg clonidine; 1 μg CRF; 10 μg prazosin; both 10 μg prazosin + 1 μg CRF; 10 μg yohimbine; both 10 μg yohimbine + 1 μg CRF. Data are expressed as mean±SEM, (n=9/group). * p<0.05 compared to saline group.

Food intake in fish injected with CRF and/or dopaminergic agonists and antagonists is shown in Figure 3. CRF, SKF 38393 and quinpirole significantly reduced feeding in goldfish. Moreover, both D1 and D2 antagonists, totally blocked the food intake inhibition by CRF. The D1 antagonist by itself increased significantly feeding with respect to control fish.

Figure 4 shows DA and DOPAC hypothalamic content and the DOPAC/DA ratio after ICV administration of dopaminergic agonists and antagonist in goldfish. The ICV injection of CRF reduced significantly DA content in goldfish. A significant reduction in DA content by both D1 agonist and antagonist was also observed. However, the hypothalamic content of DOPAC and the DOPAC/DA ratio was not significantly modified by the different treatments.
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Food intake (mg) after ICV administration of 1 μl saline; 10 μg SKF 38393; 10 μg quinpirole; 1 μg CRF; 10 μg SCH 23390; both 10 μg SCH 23390 + 1 μg CRF; 10 μg sulpiride; both 10 μg sulpiride + 1 μg CRF at 2 h postinjection in goldfish (Carassius auratus). Data are expressed as mean±SEM, (n=9/group). * p<0.05, ** p<0.01 compared to saline group.

Hypothalamic content of a) dopamine, b) DOPAC and c) the DOPAC/DA ratio after ICV administration of dopaminergic agonists and antagonists alone or combined with CRF at 2 h postinjection in goldfish (Carassius auratus). 1 μl saline; 10 μg SKF 38393; 10 μg quinpirole; 1 μg CRF; 10 μg SCH 23390; both 10 μg SCH 23390 + 1 μg CRF; 10 μg sulpiride; both 10 μg sulpiride + 1 μg CRF. Data are expressed as mean±SEM, (n=9/group). * p<0.05, ** p<0.01 compared to saline group.
Discussion

It is known that \(\alpha_1\)- and \(\alpha_2\)-adrenergic receptors have antagonistic functions on feeding in mammals (21). Thus, whereas activation of \(\alpha_1\)-adrenergic receptors suppresses food intake (22,23), activation of \(\alpha_2\)-adrenergic receptors within the PVN stimulates ingestive behavior (17,24). Our results support such dual regulation of food intake also in fish. Yohimbine (\(\alpha_2\)-adrenergic antagonist), by itself, significantly reduced food intake in goldfish, whereas prazosin (\(\alpha_1\)-adrenergic antagonist) appears to have the opposite effect. Similar data have previously been reported in C. auratus (20), although some differences in statistical signification were observed. A different feeding model could explain such statistical differences, satiated fish (20) versus food deprived fish in the present study. Nevertheless, the same trends in both experiments were found, that is, the \(\alpha_2\)-adrenergic antagonist stimulates food intake, while \(\alpha_1\)-adrenergic antagonist decreased it. These results could indicate a possible adrenergic activation by the endogenous norepinephrine of \(\alpha_1\) (when \(\alpha_2\)-receptors were blocked with prazosin) and \(\alpha_2\) (when \(\alpha_1\)-receptors were blocked with yohimbine) adrenoceptors. However, it can not be discarded a nonspecific effect of the antagonists on feeding.

Considering antagonistic effects of \(\alpha_1\)- and \(\alpha_2\)-adrenergic receptors on feeding, it would be expected opposite effects after administration of their respective agonists. However, both \(\alpha_1\) and \(\alpha_2\) agonists (phenylephrine and clonidine, respectively) at the doses used in this study did not significantly alter food intake in goldfish. Ineffective doses of the agonists and/or a possible unspecificity of these agonists in teleosts could explain this lack of effect of \(\alpha\)-adrenergic agonists on feeding. Nevertheless, the study of alteration in food intake after catecholamines administration in fish would be necessary to elucidate this subject.

Our data concerning the blockade of the CRF-induced feeding inhibition by \(\alpha_1\) antagonist allow us to suggest, for the first time, that the CRF anorectic action is mediated via \(\alpha_1\)-adrenergic receptors, at least in goldfish. To date, the exact mechanism governing the anorectic effect of CRF has not been described. Wellman et al (21) have proposed that tonicallly activated cells in the PVN inhibit feeding via \(\alpha_1\)-adrenoceptors, and the stimulation of \(\alpha_2\) receptors inactivates these tonically-inhibitory cells, thereby increasing food intake. Thus, the effect of NE on feeding can be modulated by the relative balance of these adrenergic receptors. Assuming this model, at least two hypothesis can be considered. On one hand, since CRF reduced hypothalamic NE content, it can be presumed that it only would exist the tonic activation of \(\alpha_1\)-adrenoceptors, supporting the role of this receptor subtype as mediator of the CRF anorectic action. Alternatively, it is also possible that CRF, even though decreasing hypothalamic NE, determines a greater balance of \(\alpha_2\)-adrenoceptors activity over \(\alpha_1\)-adrenoceptor activity, which would reduce food intake. Further studies should be performed to clarify the mechanism of action of NE as mediator of food intake regulation by CRF.

The role of dopamine on feeding behavior appears controversial in the literature (4,17,25). Several factors, such as the brain area (e.g. anorectic action in perifornical hypothalamus and stimulatory action in lateral hypothalamus) and/or dopamine doses (low doses enhanced feeding and higher doses inhibited it) have been shown as relevant for the response. Nevertheless, it is generally accepted an inhibitory role of DA on feeding (4,17,26,27). Our data after ICV administration of both, \(D_1\) and \(D_2\) agonists, SKF-38393 and quinpirole, respectively, support that hypothesis also in fish. Our results are in agreement with previous studies in rats, where injections of both general (28) and specific \(D_1\) and \(D_2\) (25,29,30) agonists decrease food intake. On the other hand, whereas \(D_1\) antagonist by itself showed opposite actions to that of \(D_2\) agonist, the \(D_1\) antagonist did not exert significant effect in goldfish. Similar results have been previously reported in rat, a feeding increase by \(D_1\) receptor blockade, whereas the specific \(D_1\) antagonist, SCH 23390, had not effect on food intake (31). The role of DA as mediator of CRF-induced feeding reduction in goldfish raises from the concomitant decrease in both food intake and hypothalamic DA content after ICV administration of CRF, being both responses reversed by the non-specific CRF antagonist, \(\alpha\)-Helical CRF

\(\alpha_1\)- and \(\alpha_2\)-dopaminergic antagonists (SCH 23390 and sulpiride, respectively) demonstrates the specific involvement of dopaminergic system in this inhibition of ingestive behavior in
At present, the exact mechanism of this CRF-DA interaction on feeding regulation in fish remains unknown.

Similarly to previous findings in goldfish (15), CRF reduced NE and DA hypothalamic content in the present study. Moreover, no significant modifications in the metabolites (MHPG and DOPAC, respectively) and ratios were observed. Considering the MHPG/NE and DOPAC/DA ratios as indexes of catecholamines metabolism, it appears that CRF-evoked NE and DA reduction can be due to a reduction in the rate of synthesis rather than an increase in their metabolism. In contrast, other studies conducted in rats have found that CRF increased MHPG and DOPAC (1,3). This discrepancy can be explained by the differences in experimental approaches. The studies performed in rats monitored extracellular concentrations of catecholamines in particular brain regions by in vivo microdialysis, while we measured total content of catecholamines in the whole hypothalamus. On the other hand, NE and DA reduction could be a direct consequence of food intake reduction. This last possibility is supported by previous studies in rats, which evidenced a concomitant reduction in food intake and extracellular NE in the PVN (32).

Particular mechanisms for explaining the reduction in hypothalamic DA content after D₄ agonist and antagonist injections are unknown at present. Sloley and coworkers (33) have suggested a variety of alternatives to justify the reduction of pituitary DA by domperidone (D₂-antagonist) in goldfish. On one hand, a possible interference with synthesis, transport or activity of tyrosine hydroxylase and/or another enzymes involved in the synthesis and/or degradation of catecholamines can be produced by these drugs. On the other hand, alterations in neurotransmitter reuptake and storage can occur (33). Finally, these drugs could be acting via presynaptic receptors, modifying the negative feedback on neurotransmitter synthesis and release (34). In this sense, it has been shown an increase in extracellular NE evoked by D₂-adrenoceptor blockers in rat (34), as a consequence of the inhibition of NE turnover. However, we did not observed such NE increase after yohimbine treatment (α₂ antagonist) in goldfish. These different results could be justified by the different methodological approaches used (microdialysis in rat versus quantification of total content of catecholamines in the whole hypothalamus in goldfish).

In conclusion, our results support a CRF-catecholamines interaction at central level in the control of feeding behavior in fish. Particularly, we suggest that hypothalamic α₂-adrenergic and dopaminergic systems mediate the inhibitory effect of CRF on food intake in goldfish.

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References