Mu-Opioid Receptor Is Involved in β-Endorphin-Induced Feeding in Goldfish

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THE role of the endogenous opioid system in the regulation of ingestive behavior is well established (5,22,25). The general theme in these studies shows that opioid agonists increase food intake, whereas opioid receptor antagonists decrease consumption (8,22,25,26).

Most of these reports about mediation of feeding by opioids have been performed in mammals, and some experimental approaches have also been performed in birds (12,15) and even in invertebrate species, such as slugs (20,39). Recently, studies carried out in our laboratory demonstrated for the first time that an opioid (β-endorphin) is involved in the feeding central regulation in teleosts (14). Particularly, we reported that ICV administration of β-endorphin stimulates food intake in goldfish at 2 h postinjection (14), in agreement with a number of previous studies in rats, where this endogenous opioid increases consumption after central administration (19,22,24,25).

To date, opioid receptors have been classified into mu, delta, and kappa receptor subtypes (35,36,38). These subtypes have been further divided into mu1, mu2, delta, kappa1, kappa2, and kappa3, binding sites on the basis of competitive binding and pharmacological studies in mammals (16,29).

The involvement of endogenous opioid peptides on feeding has been demonstrated by the use of opioid receptors blockers. For example, the nonselective opioid antagonist naltrexone usually attenuated food intake in a wide range of feeding situations and species (14,15,17,22,25,26,40). Nevertheless, the failure of this general opioid antagonist to distinguish among mu, delta, and kappa receptor subtypes (34) makes it difficult to identify the opioid receptor subtype involved in the feeding regulation (8,22,25,26).

Selective centrally acting antagonists of opioid receptor subtypes have been developed in the last decade, including nor-binaltorphamine (nor-BNI), a reversible kappa and particularly kappa2 antagonist (32), 7-benzidilidenenaltrexone (BNTX), a delta3 antagonist (33), naltriben, a delta2 antagonist (37), β-funtrexaamine (β-FNA), an irreversible mu antagonist (31), and naloxonazine, an irreversible mu, antagonist (30). The use of these antagonists has facilitated the understanding of the role of different opioid receptor subtypes in feeding behavior in mammals.

The present experiments were conducted to measure the effects of different opioid agonists and antagonists on feeding in goldfish, and mainly to identify the particular receptor subtype(s) involved in β-endorphin-induced feeding in the same species. Thus, on one hand, the effects of ICV administration of high-affinity, receptor-selective agonists and antagonists for mu, delta, and kappa receptors on food intake were analyzed. On the other hand, we evaluated the ability of these antagonists to block the stimulatory effect of β-endorphin on feeding.

METHOD

Animals

Goldfish (Carassius auratus). 4.52 ± 1.41 g b.wt.; Interzoo, Madrid) were maintained under natural photoperiod in 100-l
Hamilton microsyringe with a 18 Venocath cannula. The ICV the ventricular regions of the fish brain was previously established (13).

Fish were divided in seven groups (n = 7-8/group), which received two ICV injections separated by 30 min. Treatment 1: two injections of 1 μl saline; treatment 2: 1 μl saline injection followed by 1 μg injection of delta agonist (DPEN); treatment 3: 1 μl saline injection followed by β-endorphin (1 μg); treatment 4: 1 μl saline injection followed by 5 μg injection of delta antagonist (BNTX); treatment 5: BNTX (5 μg) injection followed by β-endorphin (1 μg) injection; treatment 6: saline injection followed by 5 μg injection of delta antagonist (naltriben); and treatment 7: naltriben (5 μg) injection followed by β-endorphin (1 μg) injection.

Involvement of mu receptors in the feeding regulation. Fish were divided in seven groups (n = 6–7/group) and ICV injected (two injections separated by 30 min). Treatment 1: two sequential injections of 1 μl saline; treatment 2: 1 μl saline injection followed by 1 μg injection of mu agonist (DAMGO); treatment 3: 1 μl saline injection followed by β-endorphin (1 μg); treatment 4: 5 μg injection of mu (mu1 + mu2) antagonist (B-FNA) 22 h prior to 1 μl saline injection; treatment 5: B-FNA (5 μg) injection 22 h prior to the β-endorphin (1 μg) injection; treatment 6: saline injection followed by the mu antagonist naloxonazine (5 μg) injection; and treatment 7: naloxonazine (5 μg) injection followed by β-endorphin (1 μg) injection. The 22-h interval guarantees an irreversible blockade of mu receptors (3,31).

Two ICV injections separated by 30 min. Treatment 1: two injections of 1 μl saline; treatment 2: 1 μl saline injection followed by 1 μg injection of delta agonist (DPEN); treatment 3: 1 μl saline injection followed by β-endorphin (1 μg); treatment 4: 1 μl saline injection followed by 5 μg injection of delta antagonist (BNTX); treatment 5: BNTX (5 μg) injection followed by β-endorphin (1 μg) injection; treatment 6: saline injection followed by 5 μg injection of delta antagonist (naltriben); and treatment 7: naltriben (5 μg) injection followed by β-endorphin (1 μg) injection.

Involvement of kappa receptors in the feeding regulation. Fish were divided in five groups (n = 7–8/group), which received two ICV injections separated by 30 min. Treatment 1: two injections of 1 μl saline; treatment 2: 1 μl saline injection followed by 1 μg injection of kappa agonist (DPEN); treatment 3: 1 μl saline injection followed by β-endorphin (1 μg); treatment 4: 1 μl saline injection followed by 5 μg injection of kappa antagonist (nor-BNI); treatment 5: nor-BNI (5 μg) injection followed by β-endorphin (1 μg) injection.

Two ICV injections separated by 30 min. Treatment 1: two injections of 1 μl saline; treatment 2: 1 μl saline injection followed by 1 μg injection of kappa agonist (DPEN); treatment 3: 1 μl saline injection followed by β-endorphin (1 μg); treatment 4: 1 μl saline injection followed by 5 μg injection of kappa antagonist (nor-BNI); treatment 5: nor-BNI (5 μg) injection followed by β-endorphin (1 μg) injection.

Involvement of delta receptors in the feeding regulation. Fish were divided in seven groups (n = 7–8/group), which received two ICV injections separated by 30 min. Treatment 1: two injections of 1 μl saline; treatment 2: 1 μl saline injection followed by 1 μg injection of delta agonist (DPEN); treatment 3: 1 μl saline injection followed by β-endorphin (1 μg); treatment 4: 1 μl saline injection followed by 5 μg injection of delta antagonist (BNTX); treatment 5: BNTX (5 μg) injection followed by β-endorphin (1 μg) injection; treatment 6: saline injection followed by 5 μg injection of delta antagonist (naltriben); and treatment 7: naltriben (5 μg) injection followed by β-endorphin (1 μg) injection.

Involvement of mu receptors in the feeding regulation. Fish were divided in seven groups (n = 6–7/group) and ICV injected (two injections separated by 30 min). Treatment 1: two sequential injections of 1 μl saline; treatment 2: 1 μl saline injection followed by 1 μg injection of mu agonist (DAMGO); treatment 3: 1 μl saline injection followed by β-endorphin (1 μg); treatment 4: 5 μg injection of mu (mu1 + mu2) antagonist (B-FNA) 22 h prior to 1 μl saline injection; treatment 5: B-FNA (5 μg) injection 22 h prior to the β-endorphin (1 μg) injection; treatment 6: saline injection followed by the mu antagonist naloxonazine (5 μg) injection; and treatment 7: naloxonazine (5 μg) injection followed by β-endorphin (1 μg) injection. The 22-h interval guarantees an irreversible blockade of mu receptors (3,31).

Statistical Analysis

Data were analyzed by an ANOVA test followed by Duncan's multiple range test for multigroup comparisons. A probability level of p < 0.05 was considered statistically significant.

RESULTS

Figure 1 shows the effects of agonist and antagonist for kappa receptors on feeding in goldfish, F(4, 31) = 8.1417, p < 0.0001. The ICV administration of both β-endorphin and U-50488 (kappa agonist) significantly increased food intake (p < 0.01 and p < 0.05, respectively) in goldfish (Fig. 1). The same figure shows that the kappa antagonist, nor-BNI, by itself did not alter feeding behavior in goldfish, and it was not able to counteract the β-endorphin stimulatory action on food consumption.

Figure 2 summarizes the role of delta receptors as mediators of β-endorphin effect on feeding in goldfish, F(6, 42) = 5.1211, p < 0.0005. As it can be observed, β-endorphin (p < 0.01) and the delta agonist DPEN (p < 0.05) stimulated the food intake at
administration of nor-BNI (kappa antagonist), BNTX (delta antagonist), naltrixen (delta2 antagonist), B-FNA (mu antagonist), or naloxonazine (mu antagonist), by themselves, did not affect feeding behavior in goldfish. In fact, we found food intake values similar or slightly lower than that observed in the control groups. These results are consistent with previous reports in mammals, where specific antagonists for the different opioid receptor subtypes do not produce significantly modifications of food intake or reduce ingestion, as it has been shown with the kappa antagonist nor-BNI (2.37–9.11,21,22,28), the delta antagonist DALCE (4.7–9.21), the delta antagonist naltrindol (8.9,11.21,28), the mu antagonist B-FNA (3.7–9.21), and the mu antagonist naloxonazine (7–9.21).

The present study provides new evidence for a central role of opioids on feeding regulation in poikilotherms, particularly in teleost fish. Our results about the effect of opioid agonists and antagonists on food intake in goldfish support the general agreement in homeotherms that administration of opioid agonists in the central nervous system (CNS) stimulates food intake, whereas opioid receptor antagonists reduce feeding or do not produce significant modifications in feeding (8.9,21.22.25,26,28).

Central injection of kappa (nor-BNI) and delta (BNTX, delta2, nitriben, delta2) antagonists do not counteract beta-endorphin-induced feeding behavior in goldfish. In contrast, this food intake stimulation by the opioid beta-endorphin is blocked by both beta-FNA (general mu antagonist) and naloxonazine (mu antagonist), suggesting that this stimulation of feeding is mediated via mu-opioidergic receptors in fish, particularly through the mu1 subtype. However, the blockade by beta-FNA (mu1 + mu2) could indicate an antagonism of either mu1 or mu2, or even both receptor subtypes. Thus, a possible role of the mu2 opioid receptor in the central regulation of feeding by beta-endorphin in goldfish cannot be ruled out. Unfortunately, the lack of a high-affinity, selective antagonist commercially available for mu2 receptors does not allow us to demonstrate the possible role of mu2 opioid receptors as mediators of beta-endorphin-induced feeding in goldfish.

On one hand, beta-endorphin is an endogenous mu and delta receptor ligand (1). On the other hand, binding studies using selective radioligands have identified mu and kappa receptors in goldfish brain, but neither delta nor kappa sites can be detected (10). These studies, together with the present results on the inability of kappa and delta antagonists to counteract the feeding increase by beta-endorphin, lead us to discard kappa and delta receptors as mediators of this beta-endorphin effect in goldfish.

In summary, the results presented in this article suggest that beta-endorphin-induced feeding in goldfish is mediated via mu receptors, involving, at least, the mu1 opioid receptor subtype.

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