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OPIOID SYSTEMS IN THE LATERAL HYPOTHALAMUS REGULATE FEEDING BEHAVIOR THROUGH OREXIN AND GABA NEURONS

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Abstract—The hypothalamus controls feeding behavior. Since central opioid systems may regulate feeding behavior, we examined the role of μ-, δ- and κ-opioid receptors in the lateral hypothalamus (LH), the hunger center, in feeding behavior of mice. Non-selective (naloxone; 3 mg/kg, s.c.) and selective μ- (β-funaltrexamine, β-FNA; 10 mg/kg, s.c.), δ- (naltrindole; 3 mg/kg, s.c.) and κ- (norbinaltorphimine, norBNI; 20 mg/kg, s.c.) opioid receptor antagonists significantly decreased food intake in food-deprived mice. The injection of naloxone (20 μg/side) into the LH significantly decreased food intake whereas the injection of naloxone (20 μg/side) outside of the LH did not affect food intake. The injection of β-FNA (2 μg/side), naltrindole (1 μg/side) or norBNI (2 μg/side) into the LH significantly decreased food intake. Furthermore, all these antagonists significantly decreased the mRNA level of preproorexin, but not those of other hypothalamic neuropeptides. In addition, the injection of the $GABA_A$ receptor agonist muscimol (5 μg/side) into the LH significantly decreased food intake, and this effect was abolished by the $GABA_A$ receptor antagonist bicuculline (50 μg/side). Muscimol (1 mg/kg, i.p.) decreased the mRNA level of preproorexin in the hypothalamus. Naloxone (3 mg/kg, s.c.) significantly increased the GABA level in the LH and both bicuculline and the GABA release inhibitor 3-mercaptopropionic acid (3-MP, 5 μg/side) attenuated the inhibitory effect of naloxone on feeding behavior. 3-MP also attenuated the effects of β-FNA and norBNI, but not that of naltrindole. These results show that opioid systems in the LH regulate feeding behavior through orexin neurons. Moreover, μ- and κ-, but not δ-, opioid receptor antagonists inhibit feeding behavior by activating GABA neurons in the LH.

Key words: feeding behavior, lateral hypothalamus, opioid, orexin, GABA.

INTRODUCTION

Central opioid systems have been considered to be important in the regulation of feeding behavior (Bodnar, 2007). Many reports have shown that opioid systems regulate the intake of palatable food through mesolimbic dopamine neurons that project from the ventral tegmental area (VTA) to the nucleus accumbens (NAcc) (Tanda and Di Chiara, 1998; Zhang et al., 1998; Katsuura and Taha, 2010). On the other hand, since opioid receptors are widely distributed in the brain (Mansour et al., 1995; Le Merrer et al., 2009), it is possible that an opioid system other than that in the mesolimbic area may also regulate feeding behavior. However, the evidence that other opioid systems regulate feeding behavior is limited.

The hypothalamus is a vital brain area for the control of essential feeding behavior. Since opioid receptors have been reported to be located in the hypothalamus (Mansour et al., 1995; Le Merrer et al., 2009), it is possible that opioid receptors in the hypothalamus play an important role in feeding behavior. Among specific areas in the hypothalamus, the lateral hypothalamus (LH) is classically known as the hunger center, whereas the ventromedial nucleus of the hypothalamus (VMH) is known as the satiety center (Hoebel and Teitelbaum, 1962; Grossman et al., 1978). More recently, it has been reported that some orexigenic and anorexigenic neurons are distinctly located in the LH, indicating the critical role of the LH in the regulation of feeding behavior (Brown et al., 2015). We have recently shown that the injection of opioid receptor antagonists into the LH, but not the VMH, inhibits food intake in mice (Ikeda et al., 2015a). Thus, the inhibition of opioid receptors in the LH may suppress feeding behavior.

Considerable evidence indicates that hypothalamic neuropeptides play an important role in the regulation of

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Abbreviations: 3-MP, 3-mercaptopropionic acid; AgRP, agouti-related peptide; ANOVA, analysis of variance; LH, lateral hypothalamus; MCH, melanin-concentrating hormone; NAcc, nucleus accumbens; norBNI, norbinaltorphimine; NPY, neuropeptide Y; POMC, proopiomelanocortin; PPORX, preproorexin; Pro-MCH, pro-melanin-concentrating hormone; RT-PCR, reverse transcription-polymerase chain reaction; VMH, ventromedial nucleus of the hypothalamus; VTA, ventral tegmental area; α-MSH, α-melanocyte-stimulating hormone; β-FNA, β-funaltrexamine.

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feeding behavior. For instance, agouti-related peptide (AgRP) and neuropeptide Y (NPY) are known as orexigenic neuropeptides, which stimulate food intake. Neurons that contain these neuropeptides project from the hypothalamic arcuate nucleus to many areas in the hypothalamus including the LH. α-Melanocyte-stimulating hormone (α-MSH), the cleavage product of proopiomelanocortin (POMC), is an anorexigenic neuropeptide, which inhibits food intake (Leibowitz and Wortley, 2004; Parker and Bloom, 2012). Orexin and melanin-concentrating hormone (MCH) are orexigenic neuropeptides, and neurons that contain these neuropeptides project from the LH to many brain areas outside of the hypothalamus (Rossi et al., 1997; Peyron et al., 1998; Sakurai et al., 1998). Since the inhibition of opioid receptors in the hypothalamus suppresses feeding behavior (Ikeda et al., 2015a), it is possible that opioid systems in the hypothalamus regulate feeding behavior through these neuropeptides.

In several brain areas, opioid systems have been reported to regulate various physiological functions through GABA neurons. For instance, the activation of opioid receptors increases the activity of dopamine neurons by inhibiting GABAergic interneurons in the VTA (Johnson and North, 1992; Narita et al., 2001). In addition, the activation of μ-opioid receptors inhibits GABA release from periaqueductal gray neurons, which play an important role in descending pain modulation (Hahm et al., 2004). Since GABA neurons exist in the LH (Stanley et al., 2011; Karnani et al., 2013), it is possible that GABA functions in the hypothalamus are involved in the regulation of feeding behavior by opioid systems.

Thus, the purpose of the present study was to investigate the mechanisms by which opioid systems in the LH regulate feeding behavior. As indicated above, we have recently reported that the inhibition of opioid receptors in the LH suppresses food intake (Ikeda et al., 2015a). To examine the inhibitory effects of opioid receptor antagonists on feeding behavior, we conducted the experiments under fasted condition. The reason why we conducted the experiments under fasted condition is that it is good to examine the inhibitory effects of drugs on food intake since fasted mice must eat more food than satiated mice. In fact, food deprivation-induced feeding behavior paradigm has been widely used to demonstrate the inhibition of food intake by certain drugs (Bodnar et al., 1995; Li et al., 2015). At first, we confirmed that the injection of μ-, δ- and κ-opioid receptor antagonists either systemically or into the LH inhibits feeding behavior. In addition, we investigated whether hypothalamic neuropeptides are modulated by opioid systems. Finally, we examined the possible involvement of GABA neurons in the regulation of feeding behavior by opioid receptors in the LH.

EXPERIMENTAL PROCEDURES

Animals

Male ICR mice (6–7 weeks old: 28–36 g; Tokyo Laboratory Animals Science, Tokyo, Japan) were housed in clear polycarbonate cages (20 cm × 30 cm × 15 cm) that were kept under a 12-h light/dark cycle (light on at 08:00) at a constant room temperature (24 ± 1 °C) with *ad libitum* access to regular chow diet and water. In the refeeding test, mice were deprived of food for 16 h before the experiment. Other experiments were conducted without food deprivation.

This study was carried out in accordance with the guidelines for the care and use of laboratory animals of Hoshi University, which is accredited by the Ministry of Education, Culture, Sports, Science, and Technology of Japan. All efforts were made to minimize animal suffering and to reduce the number of animals used. Each animal was used only once.

Drugs

The drugs used in the present study were the non-selective opioid receptor antagonist naloxone hydrochloride (Sigma-Aldrich, St Louis, MO, USA), the selective μ-opioid receptor antagonist β-funaltrexamine (β-FNA; (E)-4-[[(5α,6β)-17-(cyclopropylmethyl)-4,5-epoxy-3,14-dihydroxymorphinan-6-yl]amino]-4-oxo-2-butenoic acid methyl ester hydrochloride), the selective δ-opioid receptor antagonist naltrindole (17-(cyclopropylmethyl)-6,7-dehydro-4,5α-epoxy-3,14-dihydroxy-6,7-2′,3′-indolomorphinan hydrochloride), the selective κ-opioid receptor antagonist norbinaltorphimine (norBNI; 17,17′-(dicyclopropylmethyl)-6,6′,7,7′-6,6′-imino-7,7′-binorphinan-3,4′,14,14′-tetrol dihydrochloride), the $GABA_A$ receptor agonist muscimol (Sigma-Aldrich), the $GABA_A$ receptor antagonist bicuculline (Sigma-Aldrich), and the GABA synthesis and release inhibitor 3-mercaptopropionic acid (3-MP; Sigma-Aldrich). β-FNA, naltrindole and norBNI were synthesized by us (Lipkowski et al., 1986; Nagase and Fujii, 2011a; Nagase and Fujii, 2011b). β-FNA for intracerebral injection was dissolved in saline (0.9 w/v% NaCl solution) containing 20% dimethyl sulfoxide. Other drugs were dissolved in saline immediately before use. β-FNA and norBNI were injected 24 h before the refeeding test, according to previous reports that the antagonizing effects of β-FNA and norBNI appeared 24 h after systemic and intracerebral injection (Endoh et al., 1992; Suzuki et al., 1993; Zhang et al., 2007; Navratilova et al., 2015). Other drugs were injected just before the refeeding test. The doses of these drugs have been previously found to be effective on various behaviors (Grevert and Goldstein, 1977; Endoh et al., 1992; Suzuki et al., 1993; Suzuki et al., 1997) and were optimized to avoid their influence on locomotor activity.

Surgery

The surgical methods have been described previously (Ikeda et al., 2013; Ikeda et al., 2015a; Ikeda et al., 2015b). Briefly, the mice were anesthetized with sodium pentobarbital (60 mg/kg, i.p.) and stereotaxically (Narishige, Tokyo, Japan) implanted with stainless-steel guide cannulas (for microinjection: 0.5 mm o.d., 0.3 mm i.d., 4.0 mm length; for microdialysis: AG-6; Eicom, Kyoto, Japan) into the LH (A 2.58 mm, V 0.80 mm, L 1.10 mm, from the interaural line) according to the atlas of Paxinos and Franklin (2001). The cannulas were secured to the skull with a stainless screw and dental acrylic