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BRAIN RESEARCH

Research Report

A naloxonazine sensitive (μ_1 receptor) mechanism in the parabrachial nucleus modulates eating

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ABSTRACT

The parabrachial nucleus (PBN) is an area of the brain stem that controls eating and contains endogenous opioids and their receptors. Previously, we demonstrated that acute activation of μ opioid receptors (MOPR) in the lateral PBN increased food consumption. MOPRs have been divided operationally into μ_1 and μ_2 receptor subtypes on the basis of the ability of naloxonazine (Nlxz) to block the former but not the latter. We used autoradiography to measure whether Nlxz blocks stimulation by the μ_1/μ_2 agonist DAMGO (D-Ala2, N-Me-Phe4, Gly5-ol-enkephalin) of the incorporation of [^{35}S]-guanosine 5′(γ-thio)triphosphate ([^{35}S]-GTPγS) into sections of the PBN. In vitro, Nlxz dose dependently inhibited receptor coupling in all areas of the PBN. The 1 μM concentration of Nlxz reduced stimulation by 93.1±5% in the lateral inferior PBN (LPBNi) and by 90.5±4% in the medial parabrachial subregion (MPBN). Administration of Nlxz directly into the LPBNi decreased both food intake and agonist stimulated coupling, ex vivo, for the 24-h period after infusion. Infusion of Nlxz into the intended area reduced food intake by 42.3% below baseline values. Nlxz infusion prevented DAMGO stimulation of G-protein coupling in LPBNi and markedly reduced this stimulation in the MPBN. The incomplete inhibition of DAMGO-stimulated coupling in the MPBN is most likely due to the limited diffusion of Nlxz from the site of infusion (LPBNi) into this brain region. In conclusion, this study demonstrates that the μ_1 opioid receptor subtype is present in the parabrachial nucleus of the pons and that these receptors serve to modulate feeding in rats.

1. Introduction

Mu opioid receptors (MOPR) in the brain mediate physiological processes that increase feeding. Initial evidence suggesting this function for MOPRs came from studies in which administering exogenous agonists, particularly morphine and the peptide DAMGO (D-Ala2, N-Me-Phe4, Gly5-ol-enkephalin) into the nucleus accumbens (NAC) and other sites in the forebrain, increased food intake in rats (Bakshi and Kelley, 1993; Giraudo et al., 1998; Mann et al., 1988b; Ragnauth et al., 2000; Zhang and Kelley, 2000). Subsequent data demonstrated that infusing DAMGO into the nucleus tractus solitarius (NTS) (Kotz et al., 1997) also increased food intake and led to the view that MOPRs along the neuraxis, from the deep brainstem to the forebrain, code an opioidergic network that modulates feeding (Glass et al., 1999; Kelley et al., 2005; Levine, 2006).

We have shown that the pontine parabrachial nucleus (PBN) plays a significant, possibly coordinating, role in this network. The PBN relays visceral and gustatory information from the NTS and the spinal cord to the forebrain (Herbert

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et al., 1990; Hermann and Rogers, 1985; Karimnamazi et al., 2002; Lundy and Norgren, 2005). Discrete infusion of DAMGO into the lateral PBN (LPBN) increased consumption of food and this action was prevented by the nonselective opioid receptor antagonist, naloxone, and also by the selective, competitive MOPR antagonist CTAP (D-Phe-Cys-Tyr-D-Trp-Arg-Thr-Pen-Thr-NH_2) and the selective, irreversible MOPR antagonist β-FNA (β-funaltrexamine) (Ward and Simansky, 2006a, 2006b; Wilson et al., 2003). Furthermore, each of these antagonists, by themselves, reduced food intake after infusion into the LPBN. By mapping the capacity of MOPRs to couple to their G-proteins after the irreversible β-FNA, we implicated the lateral inferior subregion (LPBNi) of this area in controlling food intake (Ward and Simansky, 2006a, 2006b).

These data clearly implicated parabrachial MOPRs in the physiological control of eating. However, pharmacological evidence suggests that functional subtypes of MOPRs exist. Specifically, whereas CTAP and β-FNA antagonize all DAMGO-stimulated actions thought to involve MOPRs, the drug naloxonazine (Nlxz) appears to inhibit only some of the effects of this agonist. Those Nlxz-sensitive actions have been used operationally to define a μ_1 subtype of MOPRs (Hahn et al., 1982; Hahn and Pasternak, 1982; Ling et al., 1986; Wolozin and Pasternak, 1981). Moreover, molecular studies have also suggested the existence of subtypes of MOPRs including an alternative splice variant named MOR1-B (Narita et al., 2003; Pan et al., 2001; Pasternak, 2001). Naloxonazine binds to MOR-1B (which contains exons 1, 2, 3 and 5), indicating that this variant may be the μ_1-receptor subtype (Zimprich et al., 1995). Further, it is relevant to know that the CXBK mice have been engineered with reduced MOR1-B mRNA and, consequently, reduced the analgesic action of endomorphin-1 (Narita et al., 2003; Goldberg et al., 1998). It would be interesting to determine whether the PBN of these mice expresses MOPRs and whether these mice would show a diminished feeding response to opioid stimulation.

The μ_1-opioid receptor subtype has been implicated in some, but not all actions of MOPRs to modulate feeding (Simone et al., 1985). Infusion of the antagonist Nlxz into the ventral tegmental area (VTA) prevented the ability of hedonically positive food-related stimuli to increase dopamine (DA) release in the NAC (Tanda and Di Chiara, 1998). Nlxz did not, however, antagonize the ability of DAMGO to stimulate feeding when this agonist was infused directly into the NAC (Ragnauth et al., 2000). Additionally, intravenous (i.v.) administration of Nlxz, by itself, reduced food intake in adult male rats (Ling et al., 1986; Mann et al., 1988a, 1988b; Simone et al., 1985).

Together, these data strongly suggest regionally specific roles for μ_1 and μ_2 (i.e.: non-μ_1) receptors in feeding. It remains to be determined, however, whether the excitatory influence of parabrachial MOPRs on food intake involves a particular receptor subclass. For this, we used autoradiography to measure whether Nlxz blocks DAMGO-stimulated incorporation of [^{35}S]-guanosine 5'(γ-thio)triphosphate ([^{35}S]-GTPγS) in coronal sections through the PBN. The action of Nlxz was evaluated first in vitro, to permit characterization of receptor coupling after access of the antagonist to all areas of the PBN. We then administered Nlxz directly into the LPBNi and measured food intake, in vivo, and agonist stimulated coupling, ex vivo, for the 24-h period after infusion of the irreversible antagonist (Hahn and Pasternak, 1982; Simone et al., 1985). We demonstrate that the μ_1 opioid receptor subtype is present in the parabrachial nucleus of the pons and it serves to control food intake in rats.

2. Results

2.1. *The selective μ_1 antagonist naloxonazine inhibited DAMGO-stimulated-[^{35}S] GTPγS incorporation in the PBN, in vitro*

Fig. 1 shows the inhibition by naloxonazine (Nlxz) of DAMGO-stimulated [^{35}S]-GTPγS incorporation in the parabrachial nucleus (PBN), in vitro. In the absence of Nlxz, the selective MOPR agonist DAMGO increased GTPγS incorporation to 195 ± 25 fmol/g above basal in the LPBNi (basal value: 140 ± 9 fmol/g) and to 166 ± 23 fmol/g above basal in the MPBN (basal value: 115 ± 15 fmol/g). Nlxz reduced DAMGO-induced stimulation in a concentration-dependent manner. The 1 μM concentration of Nlxz reduced stimulation by 93.1 ± 5% in LPBNi and by 90.5 ± 4% in the medial parabrachial subregion to values (fmol/g) that were not significantly different than basal (both p values > 0.10). The MOPRs in the LPBNi and the MPBN appear to be the μ_1 subtype as defined operationally by the ability of Nlxz to interfere with the coupling function.

2.2. *Bilateral infusion of naloxonazine into the LPBNi decreased food intake*

Twenty rats were infused bilaterally with Nlxz (8 nmol/0.5 μl per side) or saline (0.5 μl) into the LPBNi. Of the ten control rats that were infused with saline, seven were judged histologically (see Fig. 2) to have placements within the LPBNi region of the parabrachial nucleus (Saline-in group) and three outside

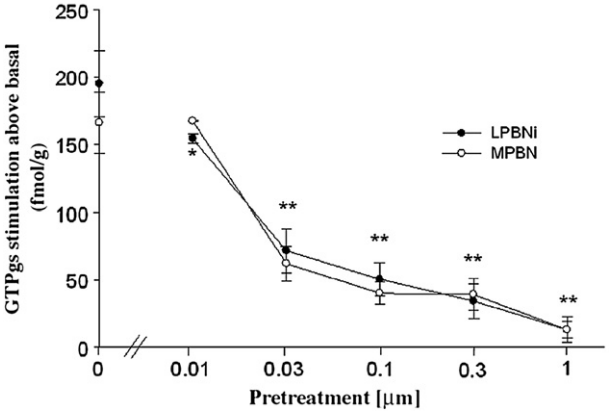

Fig. 1 – The selective μ_1 antagonist Nlxz decreased in vitro stimulation of G-protein coupling by DAMGO (1 μM) in the PBN. Data represent stimulation by DAMGO above basal values (fmol/g means ± SEM). Asterisks indicate difference from value for DAMGO without Nlxz pretreatment: $^{**}p < 0.01$ for MPBN and LPBNi, $^{*}p < 0.05$ for LPBNi ($F(5,5) = 13.7$). Student Newman–Keuls test after ANOVA ($n = 7$).