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Orphan opioid receptor antisense probes block orphanin FQ-induced hyperphagia

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Abstract

Orphanin FQ/nociceptin binds with high affinity to the orphan opioid receptor-like/κ-3 (ORL1/KOR-3) clone, and stimulates feeding. The present study demonstrated that antisense oligodeoxynucleotides directed against either exons 1, 2 or 3 of the ORL1/KOR-3 clone reduced orphanin FQ/nociceptin-induced hyperphagia. A missense probe was ineffective. Naltrexone dose-dependently reduced orphanin FQ/nociceptin-induced hyperphagia. These data suggest that the receptor responsible for orphanin FQ/nociceptin-induced hyperphagia is encoded by the ORL1/KOR-3 clone.

Keywords: Hyperphagia; Orphanin FQ/nociceptin; ORL1/KOR-3 clone

A novel receptor, termed the orphan opioid receptor-like (ORL1: e.g., Mollereau et al., 1994) clone, displays little affinity for traditional opioid peptides. This clone was homologous with an opioid clone (KOR-3) identified in our laboratory (Pan et al., 1994). Orphanin FQ (Reinscheid et al., 1995) or nociceptin (Meunier et al., 1995) binds with high affinity to the ORL1/KOR-3 clone, and has been characterized for hyperalgesic (Meunier et al., 1995; Reinscheid et al., 1995) and analgesic (Rossi et al., 1998) actions. Orphanin FQ/nociceptin, like other opioid peptides, stimulates naloxone-reversible food intake following ventricular, hypothalamic and accumbens administration (Pomonis et al., 1996; Stratford et al., 1997). The lack of a selective antagonist for the ORLl/KOR-3 receptor makes it difficult to assert that this receptor mediates orphanin FQ/nociceptin-induced hyperphagia. Antisense oligodeoxynucleotide mapping studies can evaluate whether individual exons of opioid receptor clones modulate opioid agonist-induced hyperphagia. Thus, antisense probes directed against the MOR-1 clone differentially reduce hyperphagia induced by selective μ opioid receptor agonists (Leventhal et al., 1997). Orphanin FQ/nociceptin-induced analgesia is reduced by antisense probes targeted against exons 2 or 3 of the ORL1/KOR-3 clone in rat (Rossi et al., 1998). The present study pharmacologically characterized orphanin FQ/nociceptin-induced hyperphagia using ORL1/KOR-3 antisense probes as well as central naltrexone.

Orphanin FQ/nociceptin was synthesized and purified by high-performance liquid chromatography (peptide content = 71%) with structure verified by mass spectroscopy. ORL1/KOR-3 antisense probes were synthesized (Midland) and purified for the first (Position 301–321: GGG GCA GGA AAG AGG GAC TCC), second (Position 486–505: GAC GAG GCA GTT CCC CAG GA) and third (Position 1189–1208: GGG CTG TGC AGA AGC CGA GA) coding exons. A missense probe (GGG TCG GTC AGA GAC CGA GA) for exon 3 was also employed. All sequences are specific to the ORL1/KOR-3 clone, and are not present in other opioid receptor cDNAs. All agents were dissolved in 0.9% normal saline and administered i.c.v.

As described previously (Leventhal et al., 1997), male albino Sprague–Dawley rats (90–120 days of age, Charles River Labs) were stereotaxically implanted with a guide

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cannula (22 gauge, Plastics One) into the lateral ventricle, and spontaneous food intake (6–8 h of the light cycle) was assessed following orphanin FQ/nociceptin (0, 0.5, 1, 10 nmol). Rats were evaluated for eating latency (0–15 min), and cumulative intake (0.5, 1, 2 h). Four groups received antisense or missense probes (10 μg/2 μl) on Days 1, 3 and 5. On Day 6, all rats received orphanin FQ/nociceptin (10 nmol) with intake and latency reassessed. Three additional groups received naltrexone (0.1, 1, 10 μg, Sigma) 15 min prior to orphanin FQ/nociceptin (10 nmol) with intake and latency reassessed.

Significant differences were observed among orphanin FQ/nociceptin doses after 0.5 ($F(3,33) = 17.46$, $P < 0.0001$), 1 ($F = 17.66$, $P < 0.0001$) and 2 ($F = 14.94$, $P < 0.0001$) h for intake and latency ($F = 29.71$, $P < 0.0001$). Orphanin FQ/nociceptin increased intake 3–5 fold, and decreased latency (3–5 min). Significant differences were observed among antisense treatments after 0.5 ($F(5,125) = 56.25$, $P < 0.0001$), 1 ($F = 36.23$, $P < 0.0001$) and 2 ($F = 28.93$, $P < 0.0001$) h for intake and latency ($F = 41.44$, $P < 0.0001$). Orphanin FQ/nociceptin-induced hyperphagia was significantly reduced by each of the three antisense probes (71–100%), but not by the missense control (Fig. 1A). Decreased eating latency by orphanin FQ/nociceptin was blocked by antisense, but not missense probes as well. Significant differences were observed among naltrexone doses after 0.5 ($F(4,48) = 21.81$, $P < 0.0001$), 1 ($F = 13.02$, $P < 0.0001$) and 2 ($F = 11.13$, $P < 0.0001$) h for intake and latency ($F = 27.40$, $P < 0.0001$). Orphanin FQ/nociceptin-induced hyperphagia was significantly reduced by the 10, but not the 0.1 or 1.0 μg dose of naltrexone (Fig. 1B) as was the decreased eating latency induced by orphanin FQ/nociceptin.

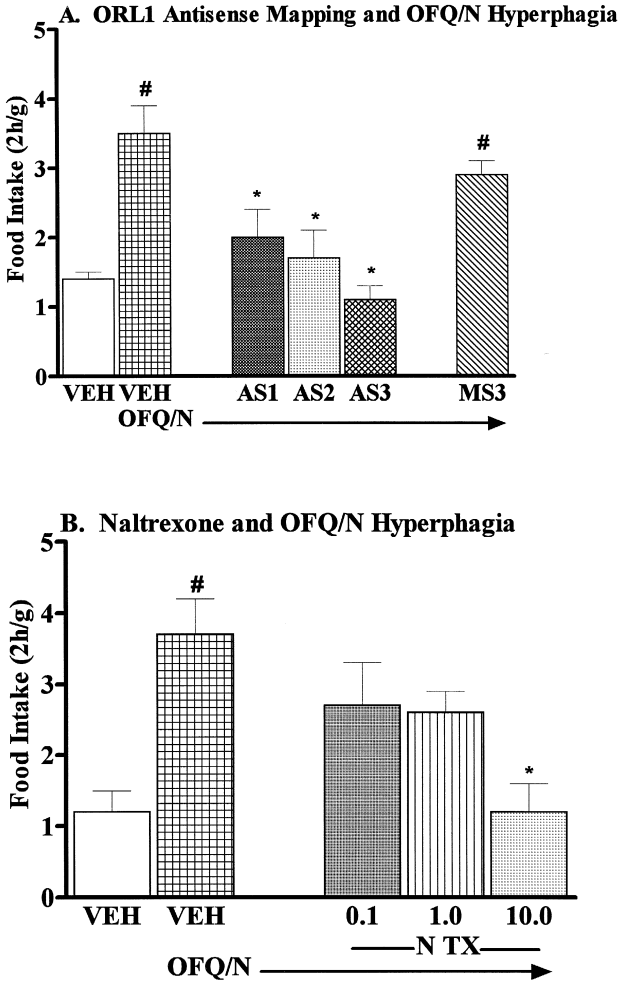

Fig. 1. Alterations in food intake (g, ±SEM) following ventricular administration of orphanin FQ/nociceptin (OFQ/N) in rats receiving either antisense oligodeoxynucleotides directed against either exons 1 (AS1), 2 (AS2) or 3 (AS3), or a missense (MS3) of the ORL1/KOR-3 clone (Panel A, $n = 6–8$/group), or ventricular naltrexone (NTX) at doses of 0.1, 1 or 10 μg (Panel B, $n = 6–7$/group). # Denotes significant increase ($P < 0.01$) in intake relative to vehicle (VEH) treatment; * Denotes significant decrease ($P < 0.01$) in intake relative to OFQ/N treatment.

Increased food intake following orphanin FQ/nociceptin and its sensitivity to naltrexone confirms previous findings (Pomonis et al., 1996; Stratford et al., 1997). Antisense mapping of the ORL1/KOR-3 clone revealed that orphanin FQ/nociceptin-induced hyperphagia is blocked by probes targeting each of the three exons. These effects are specific given the ineffectiveness of the missense control in which three pairs of bases from the antisense sequence had been switched. These data suggest that the receptor responsible for Orphanin FQ/nociceptin-induced hyperphagia is encoded by the ORL1/KOR-3 clone.

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