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Rapid communication

Naloxone sensitive orphanin FQ-induced analgesia in mice

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Abstract

Orphanin FQ, also known as nociceptin, is a heptadecapeptide with very high affinity for a novel member of the cloned opioid receptor family which produces hyperalgesia in mice. In addition to hyperalgesia, which is observed soon after administration of orphanin FQ, we now describe a delayed analgesic response. Unlike orphanin FQ-induced hyperalgesia, orphanin FQ-induced analgesia is readily reversed by the opioid antagonist naloxone, implying an opioid mechanism of action. In view of the very poor affinity of orphanin FQ for all the known traditional opioid receptors and the low affinity of opioids for the ^{125}I[Tyr^{14}]orphanin FQ binding site, orphanin FQ-induced analgesia is probably mediated through a novel orphanin FQ receptor subtype.

Keywords: Orphanin FQ; Nociceptin; Analgesia

Orphanin FQ (Reinscheid et al., 1995), also termed nociceptin (Meunier et al., 1995), is a newly identified peptide which binds with high affinity to a fourth member of the cloned opioid receptor family (ORL_1, KOR-3, LC132, XOR1, ORN7) (for reviews, see Uhl et al., 1994 and Pasternak and Standifer, 1995). It is generated from a precursor peptide which also contains two other potentially significant peptides (Meunier et al., 1995; Pan et al., 1996). Pharmacologically, orphanin FQ is quite unique. Orphanin FQ elicits hyperalgesia (Meunier et al., 1995; Reinscheid et al., 1995), an enhanced response to a nociceptive stimulus which is opposite to the analgesia produced by traditional opioids. Although we also observe orphanin FQ-induced hyperalgesia, the pharmacology of orphanin FQ now appears to be more complex.

Male CD-1 mice (25 g) were purchased from Charles River Laboratories (Raleigh, NC, USA). Naloxone was a generous gift from the Research Technology Branch of the National Institute on Drug Abuse. Orphanin FQ was synthesized in the Core Facility at Memorial Sloan-Kettering Cancer Center and purified by high-performance liquid chromatography (HPLC) with a peptide content of approximately 55%. Structure was verified by mass spectroscopy. Antinociception was assessed using the tail-flick assay. Baseline latencies, ranging from 2 to 3 s, were determined for each mouse. Analgesia was defined quantally as a doubling or greater of the baseline latency for an individual mouse. Comparisons were made using the Fisher exact test.

Hyperalgesia cannot be observed in traditional tail-flick paradigms due to the short baseline latencies. Decreasing the intensity of the stimulus lengthens the baseline latencies, which permits hyperalgesia to be detected. Using this modified paradigm, we also observe the rapid appearance within 15 min of orphanin FQ-induced hyperalgesia. However, extending the period of observation beyond 30 min results in a progressive increase in the tail-flick latencies to values well above baseline levels, suggesting a delayed analgesic effect. We therefore decided to examine orphanin FQ in a traditional tail-flick assay. Given intracerebroventricularly, orphanin FQ (10 μg i.c.v.) produces a robust and reproducible analgesia in just under 50% of the mice which peaks between 30 and 45 min after injection (Fig. 1). Orphanin FQ-induced hyperalgesia is insensitive to opioid antagonists, but orphanin FQ-induced analgesia is readily reversed by naloxone ($P < 0.04$), indicating an opioid mechanism of action.

Thus, orphanin FQ elicts both a rapid hyperalgesia and a delayed analgesia. The sensitivity of only orphanin FQ-induced analgesia to opioid antagonists indicates that the hyperalgesia and analgesia are mediated through distinct receptors. It is unlikely that orphanin FQ acts through traditional opioid receptors despite its sensitivity to nalox-

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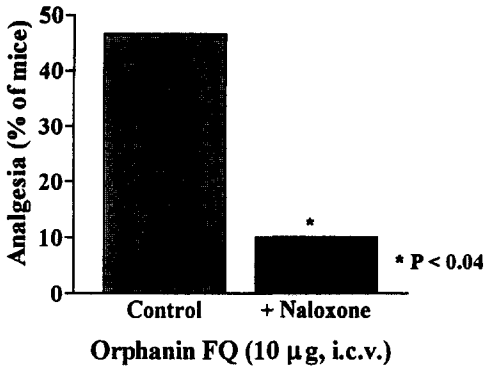

Fig. 1. Orphanin FQ analgesia in mice. Groups of mice received orphanin FQ (10 μg) alone ($n = 30$) or with naloxone (1 mg/kg s.c.) given immediately before. Analgesia was assessed quantally 45 min after the orphanin FQ. Naloxone significantly lowers the analgesic response by orphanin FQ ($P < 0.04$).

one since orphanin FQ has very poor affinity for these binding sites. Although orphanin FQ binds with very high affinity to the expressed KOR-3 receptor (Pan et al., 1994, 1995), the mouse homolog of the orphan opioid receptor, this receptor also is not likely to mediate orphanin FQ-induced analgesia since it has very poor affinity for traditional opioids such as naloxone. Thus, orphanin FQ-induced analgesia is probably mediated through a novel orphanin FQ receptor subtype. This is particularly interesting in view of the close association of the KOR-3 clone to the kappa$_3$ receptor (Pan et al., 1994, 1995; Pasternak and Standifer, 1995). The blockade of kappa$_3$ analgesia by a series of antisense probes targeting the second and third coding exons but not by five additional antisense probes based upon the first coding exon led to the suggestion that the kappa$_3$ and the KOR-3 receptors might be splice variants. The present observation of orphanin FQ-induced analgesia further supports the classification of the orphan receptor within the opioid receptor family and raises interesting questions regarding its relationship to traditional opioid analgesia.

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