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SHORT COMMUNICATION

Functional Blockade of Opioid Analgesia by Orphanin FQ/Nociceptin

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ABSTRACT. Orphanin FQ/nociceptin (OFQ/N) is a recently identified neuropeptide with high affinity for the orphan opioid receptor. OFQ/N blocked morphine analgesia in mice in a dose-dependent manner, as well as the analgesic actions of [D-Pen^2, D-Pen^5]enkephalin (DPDPE), morphine-6β-glucuronide, *trans*-3,4-dichloro-*N*-[2-(1-pyrrolindinyl)-cyclohexyl]-benzeneacetamide, methane sulfonate hydrate (U50,488H) and naloxone benzoylhydrazone. These actions are anti-analgesic, because OFQ/N also blocked clonidine analgesia and OFQ/N was inactive against the inhibition of gastrointestinal transit by morphine. Although OFQ/N was quite potent in these paradigms, two truncated forms, OFQ/N(1-11) and OFQ/N(1-7), were inactive. An antisense oligodeoxynucleotide targeting the first coding exon of KOR-3, the mouse homolog of the orphan opioid receptor, effectively prevented the anti-opioid actions of OFQ/N, confirming the importance of the orphan opioid receptor in this action. BIOCHEM PHARMACOL **55**;9:1537–1540, 1998. © 1998 Elsevier Science Inc.

KEY WORDS. morphine; opioid receptor; KOR-3; analgesia; anti-opioid

Following the cloning of the traditional opioid receptors, a novel cDNA was cloned from the mouse (KOR-3†) [1, 2] and from other species (ORL_1, LC132, ORN7, and XOR1) [3–11]. Although closely associated with the κ_3 receptor [1, 2, 12, 13], the two receptors are not the same. Two groups recently isolated an endogenous heptadecapeptide (Phe-Gly-Gly-Phe-Thr-Gly-Ala-Arg-Lys-Ser-Ala-Arg-Lys-Leu-Ala-Asp-Glu), which is structurally similar to dynorphin A and has high affinity for the KOR-3/ORL_1 receptor, termed orphanin FQ [14] or nociceptin [15]. Although in initial studies supraspinal OFQ/N was reported to produce hyperalgesia in mice [14, 15] and others have reported that OFQ/N exhibits an anti-opioid effect [16, 17], we observed a more complex pharmacology [18, 19]. OFQ/N also produces a naloxone-sensitive analgesia [18–21]. We now have explored the interactions of OFQ/N with opioid analgesia.

MATERIALS AND METHODS

OFQ/N, OFQ/N(1-7), and OFQ/N(1-11) were synthesized by the Core Facility at the Memorial Sloan-Kettering Cancer Center, purified by HPLC, and their structures verified by mass spectroscopy. Peptide contents were approximately 60%. M6G, DPDPE, and *trans*-3,4-dichloro-*N*-[2-(1-pyrrolindinyl)-cyclohexyl]-benzeneacetamide, methane sulfonate hydrate (U50,488H) were gifts from the Research Technology Branch of the National Institute on Drug Abuse. NalBzoH was synthesized as described previously [22]. Haloperidol and clonidine were purchased from the Sigma Chemical Co., and halothane was purchased from Halocarbon Laboratory.

Male CD-1 mice (25–30 g; Charles River Laboratories) were on a 12-hr light/dark cycle with food and water available *ad lib.* and were used only once. Drugs were administered intracerebroventricularly (i.c.v.) under halothane anesthesia [18, 23]. Analgesia was assessed quantally as a doubling or greater of the baseline latency for each mouse, typically between 2 and 3 sec, using the tailflick assay with baseline latencies from 2–3 sec [1, 2, 18]. To minimize tissue damage, we used a 10-sec cutoff. Comparisons were performed by the Fischer exact test.

The KOR-3 [1, 2] antisense oligodeoxynucleotides were synthesized by the Midland Certified Reagent Co., were purified in our laboratory, and were dissolved in 0.9% saline. Antisense A (GGG GCA GGA AAG AGG GAC TCC) corresponds to nucleotides 301–321 (first coding exon) and Antisense C (GGG CTG TGC AGA AGC CGA GA) corresponds to nucleotide sequences 1189–1208 (third coding exon). Because the *KOR-3* gene contains an additional upstream noncoding exon [24] that was not seen in the early cloning studies [1, 2], Antisense A and C target exons 2 and 4 of the gene, respectively. The mismatch oligodeoxynucleotide (GGG TCG GTC AGA GAC CGA GA) differs from Antisense C by only 3 pairs of bases. Mice received antisense (5 μg in 2 μL, i.c.v.)

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† *Abbreviations:* KOR-3, cDNA encoding the murine orphan opioid receptor; OFQ/N, orphanin FQ/nociceptin; M6G, morphine-6β-glucuronide; DPDPE, [D-Pen^2, D-Pen^5]enkephalin; and NalBzoH, naloxone benzoylhydrazone.

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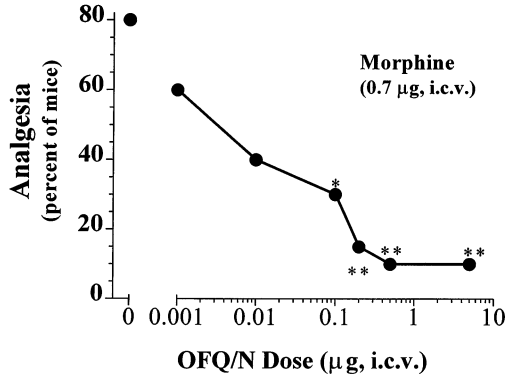

FIG. 1. Dose response of the anti-opioid effect of OFQ/N. Groups of mice ($N = 20$) received morphine (0.7 μg, i.c.v.) and the indicated dose of OFQ/N. Analgesia was assayed 15 min later. OFQ/N at 0.1 μg (*$P < 0.01$), 0.2, 0.5, and 5 μg ($P < 0.001$) significantly lowered the response.**

under halothane anesthesia on days 1, 3, and 5 and were tested on day 6, as previously described [2, 18, 25, 26].

RESULTS

In previous studies, OFQ/N reversed morphine analgesia [16, 17]. Our findings confirm these earlier results. OFQ/N lowered morphine analgesia in a dose-dependent manner with very high potency (Fig. 1). The analgesic activity of morphine was reduced by half at an OFQ/N dose of only 0.1 μg. OFQ/N (0.2 μg, i.c.v.) also attenuated M6G (12.5 ng, i.c.v.), DPDPE (8 μg, i.c.v.), U50,488H (75 μg, i.c.v.), NalBzoH (20 μg, i.c.v.), and clonidine (1 μg, i.c.v.) antinociception (Fig. 2). OFQ/N also reversed pre-existing morphine analgesia (Fig. 3). OFQ/N(1-11) is an effective analgesic with a potency similar to that of OFQ/N. OFQ/N(1-7) also is active [18]. Yet, neither OFQ/N(1-7) nor

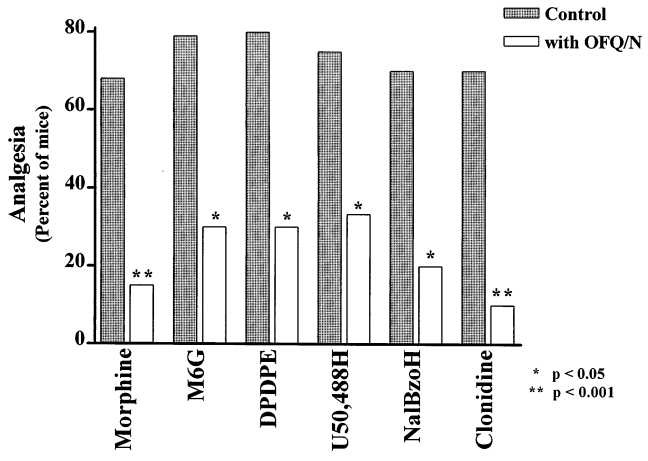

FIG. 2. Blockade of opioid and nonopioid analgesia by OFQ/N. Groups of mice ($N \geq 20$) received OFQ/N (0.2 μg, i.c.v.) or saline with either M6G (12.5 ng, i.c.v.), DPDPE (8 μg, i.c.v.), U50,488H (75 μg, i.c.v.), NalBzoH (20 μg, i.c.v.), or clonidine (1 μg, i.c.v.). Analgesia was assessed after 15 min. Under these conditions, OFQ/N alone has no observable activity.

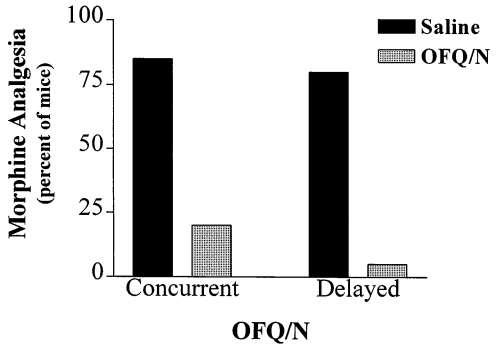

FIG. 3. Reversal of established morphine analgesia. Groups of mice ($N = 20$) received morphine (7.25 mg/kg, s.c.) at the same time as (Concurrent) or 25 min prior to OFQ/N (0.2 μg, i.c.v.; Delayed). Analgesia was assayed 15 min after OFQ/N. OFQ/N significantly lowered the response, regardless of whether the OFQ/N was given concurrently ($P < 0.001$) or 25 min after the morphine ($P < 0.001$).

OFQ/N(1-11) (1 μg, i.c.v.) affected morphine analgesia at doses 5-fold higher than an active dose of OFQ/N (Fig. 4).

In prior studies, an antisense probe targeting the first coding exon of KOR-3 blocked OFQ/N-induced hyperalgesia, but not analgesia [18]. This same antisense probe, Antisense A, prevented the reversal of morphine analgesia by OFQ/N, but Antisense C had no effect in this paradigm (Fig. 5) despite its activity against OFQ/N and NalBzoH analgesia [1, 2, 18].

DISCUSSION

OFQ/N, the recently discovered endogenous ligand for the orphan opioid receptor, was reported initially to produce hyperalgesia [14, 15]. Our own results confirmed an initial shortening of tailflick latencies, consistent with an increased sensitivity to pain, which then was followed by an analgesic response [18]. Thus, OFQ/N has a complex pharmacology and is capable of eliciting opposing actions

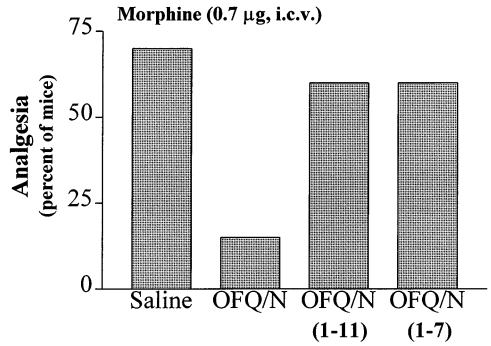

FIG. 4. Reversal of morphine analgesia by OFQ/N and its fragments. Groups of mice ($N \geq 20$) received saline, OFQ/N(1-7) (1 μg, i.c.v.), OFQ/N(1-11) (1 μg, i.c.v.), or OFQ/N (0.2 μg, i.c.v.) and morphine (0.7 μg, i.c.v.). Analgesia was assessed after 15 min. Only OFQ/N significantly lowered the morphine response ($P < 0.004$).

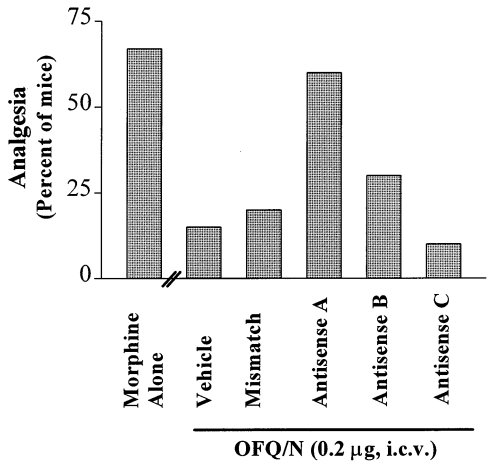

FIG. 5. Effects of KOR-3 antisense on OFQ/N reversal of morphine analgesia. Groups of mice ($N \geq 10$) received saline or the indicated oligodeoxynucleotide (10 μg, i.c.v.) on days 1, 3, and 5. On day 6, all mice were given OFQ/N (0.2 μg, i.c.v.) and morphine (0.7 μg, i.c.v.) or morphine alone, and analgesia was assessed 15 min later. Antisense A targets the first coding exon of KOR-3 and Antisense C targets the third. OFQ/N in the vehicle-treated animals significantly lowered the morphine response ($P < 0.001$). This blockade was reversed significantly by Antisense A ($P < 0.05$).

that depend upon the dose and paradigm employed, as well as the strain of mouse [18].

The recent studies demonstrating the reversal of opioid analgesia by OFQ/N [16, 17, 27] provide strong support for the concept of an anti-opioid OFQ/N system, which may prove important in explaining the initial descriptions of OFQ/N activity. In these paradigms, OFQ/N enhanced the response to nociceptive stimuli. In some situations, this reflects the reversal by OFQ/N of an opioid-mediated stress-induced antinociception [16, 17], but similar responses have been observed in a paradigm in which careful examination has not revealed any opioid-mediated stress responses [18]. Against opioid analgesia, the actions of OFQ/N are quite clear and dramatic, confirming its potent anti-opioid activity [16, 17, 27]. OFQ/N reversed morphine analgesia in a dose-dependent manner. It is particularly interesting that OFQ/N attenuates morphine analgesia far more potently than it produces hyperalgesia [18], with half-maximal responses for these two actions differing by approximately 50-fold. The anti-opioid actions of OFQ/N are not limited to morphine. Earlier studies had revealed a similar activity against δ and κ_1 opioids [16]. OFQ/N also blocks the κ_3 analgesic NalBzoH and M6G, which acts through its own receptor [28–33]. Despite its potent reversal of morphine analgesia, OFQ/N was inactive against the inhibition of gastrointestinal transit, suggesting that OFQ/N selectively attenuates analgesic systems and is not simply anti-opioid. Its reversal of clonidine further supports the concept of a selective anti-analgesic system.

All OFQ/N actions can be attenuated by antisense probes based upon KOR-3, the mouse homolog of the orphan opioid receptor. Antisense A, which targets the first KOR-3 coding exon [1, 2, 24], effectively prevented the reversal of morphine analgesia by OFQ/N, much like its effects against OFQ/N hyperalgesia [18], while Antisense C was inactive. Conversely, Antisense A was inactive against OFQ/N analgesia, which was readily blocked by Antisense C. Thus, both antisense probes can down-regulate OFQ/N actions. The different selectivity profiles of the anti-opioid and analgesic actions, however, raise the possibility that these activities involve different receptor subtypes. The actions of OFQ/N(1-11) support this concept. Although OFQ/N(1-11) is as potent as OFQ/N itself in an analgesia paradigm [18], it displayed no anti-opioid activity.

In conclusion, OFQ/N has potent anti-opioid actions that are not shared by its truncated fragments, OFQ/N(1-11) or OFQ/N(1-7). This anti-opioid action extends to all the described opioid receptor classes and nonopioid systems as well. This is not a simple anti-opioid effect since OFQ/N was inactive against the inhibition of gastrointestinal transit by morphine. Furthermore, the OFQ/N receptors responsible for this anti-opioid action are readily distinguished pharmacologically from those mediating OFQ/N analgesia. Together, these results support the complex nature of OFQ/N pharmacology [18, 34].

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