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Nociceptin/orphanin FQ blocks the antinociception induced by mu, kappa and delta opioid agonists on the cold water tail-flick test

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Abstract

Nociceptin/orphanin FQ (N/OFQ), a 17-amino-acid peptide, is an endogenous agonist whose receptor is similar in sequence to mu, delta and kappa opioid receptors. It has been reported that N/OFQ can block antinociceptive effects induced by opioid receptor agonists in the radiant heat tail-flick test and warm water tail-withdrawal test. The present studies were designed to see the effect of N/OFQ on antinociception induced by opioid receptor agonists in the cold water tail-flick (CWT) test, which measures a different type of pain. In adult male Sprague Dawley (S-D) rats given subcutaneous (s.c.) injections of saline or morphine (8 mg/kg), intracerebroventricular (i.c.v.) injection of N/OFQ (18 µg) 15 min later produced a significant reversal of subcutaneous morphine antinociception ($p < 0.01$, ANOVA followed by Duncan's test), compared to the corresponding saline control group. Saline ($t = +15$ min, i.c.v.) had no effect on s.c. morphine antinociception ($p > 0.01$), compared to the corresponding saline control group. When the kappa opioid receptor agonist spiradoline (80 mg/kg, s.c.) was used instead of morphine, similar results were observed. In another series of experiments, It was found that i.c.v. injection of N/OFQ (18 µg) reversed the antinociception induced by i.c.v. injection of the selective mu opioid agonist PL017 (2 µg), delta opioid agonist DPDPE (50 ng) and kappa opioid agonist dynorphin (21.5 µg), respectively. These results indicate that N/OFQ may be an endogenous anti-opioid peptide in the brain of rats in the CWT test.

Keywords

Orphanin FQ/Nociceptin; opioid agonists; antinociception; cold water tail-flick test; rats

1. Introduction

A seventeen-amino-acid peptide, N/OFQ, has been isolated from rat brain, and it is an endogenous agonist of the ORL₁ (Opioid-Receptor-Like) receptor, which is similar in sequence to mu, delta and kappa opioid receptors (~75% homology) (Meunier et al., 1995; Reinscheid et al., 1995). Despite many structural homologies to the opioid system, the N/OFQ receptor (NOP receptor) shows low-affinity binding to selective opioid agonists or antagonists (Meis, 2003; Mogil et al., 1996a; Mogil et al., 1996b). NOP receptors are found throughout almost all areas of the central nervous system, including spinal cord dorsal horn, nucleus raphe

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magnus, and the periaqueductal gray (Neal et al., 1999a; Neal et al., 1999b). The NOP receptor is coupled to G proteins, whose activation results in an inhibition of adenylate cyclase, modulation of calcium and potassium currents, and regulation of transmitter systems (Moran et al., 2000). N/OFQ actions include hyperalgesia, analgesia, antagonism of analgesia, allodynia, thermoregulation, anxiolytic actions, modulation of opioid-mediated processes, and influences on learning and memory (Heinricher, 2003; Shane et al., 2001; Chen et al., 2001; Jenck et al., 1997).

Some reports (Meunier et al., 1995; Reinscheid et al., 1995; Suaudeau et al., 1998) indicated that N/OFQ induced hyperalgesia when administered i.c.v. to mice in the hot-plate test. N/OFQ dose-dependently (2.5-25 nmol) reversed systemic morphine (5 mg/kg, s.c.) antinociception in mice, and 10 nmol also antagonized the antinociception induced by i.c.v. injection of DAMGO (0.01-0.1 nmol), DPDPE (10-50 nmol) and U50,488H (100-1000 nmol) in mice in the warm water tail-withdrawal test (Mogil et al., 1996b). Another report indicated that N/OFQ acts as a supraspinal, but not a spinal, anti-opioid peptide (Grisel et al., 1996). However, Xu et al (Xu et al., 1996) found that N/OFQ (1 or 10 μ g) has a potent spinal antinociceptive effect in anesthetized rats. N/OFQ can attenuate antinociception induced by opioid agonists (Citterio et al., 2000; King et al., 1998; Mogil et al., 1996a; Mogil et al., 1996b) and by acupuncture (Du et al., 1998; Meis, 2003; Wang et al., 1998; Zhu et al., 1996; Zhang et al., 1997). N/OFQ is also reported to block the analgesia produced by paracetamol, a nonopioid analgesic drug (Sandrini et al., 2005).

The cold water tail-flick (CWT) test in rats is an antinociceptive test that distinguishes opioid agonists acting on all three opioid receptor types from mixed agonist-antagonists (Pizziketti et al., 1985). To help clarify the effect of N/OFQ on analgesia, experiments were designed to determine whether there are synergistic or antagonistic interactions between N/OFQ and opioid agonists (morphine, spiradoline, PL017, DPDPE, dynorphin) in the rat CWT test.

2. Materials and Methods

2.1. Animals

Male Sprague-Dawley rats (Zivic-Miller), weighing 175-200 g, were housed in groups of 3-4 for at least 1 week in an animal room maintained at $22\pm1^{\circ}\text{C}$ and approximately $50\pm5\%$ relative humidity. Lighting was on a 12/12 h light/dark cycle (lights on at 7:00 and off at 19:00). Rats were allowed free access to food and water. All animal use procedures were conducted in strict accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and were approved by the Institutional Animal Care and Use Committee.

2.2. Surgery procedures

Animals were anesthetized with a mixture of ketamine hydrochloride (100-150 mg/kg) and acepromazine maleate (0.2 mg/kg). A cannula made of PE-10 tubing (outer diameter 0.61 mm) was implanted into the right lateral ventricle using the following stereotaxic coordinates: A 5.4, LR 1.5, H 3.5, according to Pellegrino and Cushman, system A (Pellegrino and Cushman, 1967). The animals were housed individually after surgery. Experiments began 1 week postoperatively. Each rat was used only once. At the end of the experiment, cannula placements were verified using microinjection of 1% bromobenzene blue according to the standard procedures in our laboratory (Handler et al., 1994).

2.3. Nociceptive test

The latency to flick the tail in cold water was used as the antinociceptive index, according to a standard procedure in our laboratory (Pizziketti et al., 1985). A 1:1 mix of ethylene glycol:water was maintained at -3°C with a circulating water bath (Model 9500, Fisher

Scientific; Pittsburgh, PA). Restrained in a holder with the tail protruding, rats were held over the bath with their tails submerged approximately half-way into the solution. All animals were tested at 60, 15 and 0 min before drug injection. For each animal, the first reading was discarded and the mean of the second and third readings was taken as the baseline value. Rats whose baseline values fell within a range of 10 to 20 s were used in the experiments. About 2% of them were discarded. Latencies to tail flick after injection were expressed as percentage change from baseline. The percent of maximal possible antinociception (MPA) for each animal at each time was calculated using the formula: $\%MPA = [(test\ latency - baseline\ latency)/(60 - baseline\ latency)] \times 100$. A cutoff limit of 60 s was set to avoid damage to the tail.

2.4. Drugs

The selective kappa-opioid receptor agonist spiradoline mesylate (U62,066, spiradoline), an arylacetamide, was a gift from the Upjohn Company, Kalamazoo, MI. Morphine was made by Research Triangle Institute and supplied through NIDA. N/OFQ (H-Phe-Gly-Gly-Phe-Thr-Gly-Ala-Arg-Lys-Ser-Ala-Arg-Lys-Leu-Ala-Asn-Gln-OH), PL017 (H-Tyr-Pro-(N-Me)Phe-D-Pro-NH₂), [D-Pen², D-Pen⁵]-enkephalin (DPDPE) (H-Tyr-D-Pen-Gly-Phe-D-Pen-OH) and dynorphin A (1-17) (H-Tyr-Gly-Gly-Phe-Leu-Arg-Arg-Ile-Arg-Pro-Lys-Leu-Lys-Trp-Asp-Asn-Gln-OH) were made by Multiple Peptide Systems, San Diego, CA and supplied through NIDA. All the opioid receptor agonists and N/OFQ were dissolved in the 0.9% saline.

2.5. Injections

For i.c.v. injection, N/OFQ or PL017, DPDPE or dynorphin were given 15 min after i.c.v. injection of opioid agonists or saline in a volume of 5 μ l followed by 3- μ l saline flush over 30 s. For s.c. injection, the morphine or spiradoline was given 15 min after dorsal s.c. injection of opioid agonists or saline in 10 s.

2.6. Statistical analysis

The data are expressed as the mean and standard error (S.E.M.). Statistical analysis of difference between groups was assessed with a two-way analysis of variance (ANOVA) followed by Duncan's test and with a grouped t-test. $P \leq 0.05$ was taken as the significant level of difference.

3. Results

3.1. N/OFQ blockade of antinociception induced by s.c. injection of opioid receptor agonists

In this experiment, rats were divided into 4 groups receiving a s.c. injection of morphine (8 mg/kg) or saline 15 min before i.c.v. microinjection of N/OFQ (18 μ g) or saline (Fig. 1). Previous data from our laboratory showed that i.c.v. injection of N/OFQ at doses from 0.09 μ g to 18 μ g did not induce a significant change in the CWT test. For the 4 groups, the average baseline latencies of the rats used in this experiment were as follows: 18.2 ± 0.5 s (saline + saline group), 17.6 ± 0.6 s (saline + N/OFQ group), 18.0 ± 0.7 s (morphine + saline group) and 17.3 ± 0.4 s (morphine + N/OFQ). The results showed that in rats given s.c. injections of saline or morphine (8 mg/kg), i.c.v. injection of N/OFQ (18 μ g) 15 min later produced a significant reversal of s.c. morphine antinociception ($P < 0.05$, ANOVA followed by Duncan's test), compared to the corresponding morphine + saline group. Saline ($t = +15$ min, i.c.v.) did not affect s.c. morphine antinociception. Neither saline nor N/OFQ had an effect themselves on antinociception in the CWT test.

In the next experiment, the kappa opioid receptor agonist spiradoline (80 mg/kg, s.c.) was used instead of morphine as in the previous series of experiments. Similar results were observed (Fig. 2). The average baseline response latencies were as follows: 17.9 ± 0.6 s (saline + saline group), 18.9 ± 0.4 s (saline + N/OFQ group), 17.7 ± 0.4 s (spiradoline + saline group) and 17.1

+ 0.4 s (spiradoline + N/OFQ). The data showed that N/OFQ antagonized the antinociception induced by s.c. injection of spiradoline ($P < 0.05$, compared to the corresponding spiradoline + saline group). There were no significant differences between the saline + saline group and the saline + N/OFQ group ($P > 0.05$).

3.2. N/OFQ blockade of antinociception induced by i.c.v. injection of specific opioid receptor agonists

Rats were divided into 4 groups receiving an i.c.v. injection of the mu opioid receptor agonist PL017 (2 μ g) or saline 15 min before i.c.v. microinjection of N/OFQ (18 μ g) or saline (Fig. 3). The average baseline response latencies were as follows: 17.9 + 0.4 s (saline + saline group), 17.7 + 0.4 s (saline + N/OFQ group), 18.2 + 0.2 s (PL017 + saline group) and 17.9 + 0.4 s (PL017 + N/OFQ). The results in Fig. 3 showed that i.c.v. injection of N/OFQ (18 μ g) reversed the antinociception induced by i.c.v. injection of the selective mu opioid agonist PL017 (2 μ g), although the time course is delayed, compared with those of DPDPE (Fig. 4) and dynorphin (Fig. 5).

In the next experiment, the design was similar to that in the previous one, except for substitution of the mu opioid receptor agonist PL017 by the delta opioid receptor agonist DPDPE (50 ng) (Fig. 4) or the kappa opioid receptor agonist dynorphin (21.5 μ g) (Fig. 5). The average baseline latencies for figure 4 were as follows: 17.6 + 0.4 s (saline + saline group), 18.2 + 0.4 s (saline + N/OFQ group), 17.1 + 0.3 s (DPDPE + saline group) and 18.0 + 0.3 s (DPDPE + N/OFQ); for figure 5: 18.2 + 0.4 s (saline + saline group), 18.1 + 0.5 s (saline + N/OFQ group), 18.2 + 0.4 s (dynorphin + saline group) and 18.7 + 0.3 s (dynorphin + N/OFQ). These results showed that i.c.v. injection of N/OFQ (10 nmol) reversed the antinociception induced by i.c.v. injection of selective delta and kappa opioid agonists, respectively.

4. Discussion

Morphine produces analgesia through the opioid system (mu, delta and kappa opioid receptors). Spiradoline is highly selective for the kappa receptor and easily penetrates the blood brain barrier (Wadenberg, 2003). PL017 is an opioid agonist highly selective for mu receptors and produces long-lasting, naloxone-reversible analgesia in rats (Chang et al., 1983). Dynorphin is an endogenous kappa ligand (Chavkin et al., 1982; Goldstein et al., 1979), and DPDPE is a delta opioid receptor agonist (Mosberg et al., 1983). The present results show that i.c.v. injection of N/OFQ can block antinociception induced by s.c. injection of morphine and spiradoline, and that induced by i.c.v. injection of selective mu (PL017), delta (DPDPE) or kappa (dynorphin) opioid agonists in the rat CWTtest. These findings strongly suggest that N/OFQ may be an endogenous anti-opioid peptide in the brain of rats.

There is much evidence that N/OFQ is an anti-opioid peptide at the supraspinal level. I.c.v. administration of orphanin FQ (N/OFQ) blocks opioid-induced antinociception in a variety of animal models of pain. An inhibitory effect of N/OFQ on morphine-induced antinociception using the hot-plate test in rats has also been reported (Lufty et al., 1999). Morgan et al. (1997) demonstrated that microinjection of N/OFQ into periaqueductal grey blocked the antinociceptive effects of morphine applied at the same site, suggesting that the periaqueductal grey is an important area for N/OFQ to exert an anti-opioid effect. N/OFQ-immunoreactive in the brain increased in morphine-tolerant rats, and i.c.v. N/OFQ antibody reversed morphine tolerance (Tian et al., 1998; Yuan et al., 1999). Zhu et al. (Zhu et al., 1997) reported that i.c.v. N/OFQ could aggravate formalin-induced pain behavior and attenuate morphine analgesia, whereas i.c.v. injection of antisense oligonucleotide complementary to the NOP receptor decreased the pain behavior. N/OFQ(1-17) attenuated the tail-flick inhibition produced by both morphine and kainic acid microinjection (Morgan et al., 1997). N/OFQ(1-17) reverses opioid antinociception by inhibiting periaqueductal grey output neurons, suggesting that the

functional anti-opioid effects of N/OFQ(1-17) are mediated by periaqueductal grey neurons (Morgan et al., 1997). Our experiments on thermoregulation indicated that N/OFQ probably acts as a physiological antagonist to reduce morphine-induced hyperthermia (Chen et al., 2001). Neither the opioid receptor antagonist naloxone nor the kappa opioid receptor antagonist nor-binaltorphimine reduced the hyperthermia induced by i.c.v. injection of N/OFQ.

Spinal (i.t.) or supraspinal (i.c.v.) administration of N/OFQ in mice and rats has shown an antagonistic effect of N/OFQ on opioid analgesia in different analgesic tests. Neither mice treated with N/OFQ alone, either i.t. or i.c.v., nor rats administered i.c.v. or i.t. N/OFQ displayed a significant difference in the hot-plate, warm-water or radiant heat tail-flick tests (Lufty and Maidment, 2000; Vanderah et al., 1998). N/OFQ antagonized the antinociception induced by i.c.v. injection of DAMGO (0.01-0.1 nmol), DPDPE (10-50 nmol) and U50,488H (100-1000 nmol) in mice in the warm water tail-withdrawal test (Mogil et al., 1996b). In the present study, similar results have been found in the CWT test in that i.c.v. injection of N/OFQ (18 µg) reversed the antinociception induced by i.c.v. injection of the selective mu opioid agonist PL017 (2 µg), delta opioid agonist DPDPE (50 ng) and kappa opioid agonist dynorphin (21.5 µg), respectively, in the CWT test.

These results are consistent with many reports (Bytner et al, 2001; Citterio et al., 2000; King et al., 1998; Mogil et al., 1996a; Mogil et al., 1996b; Mogil and Paternak, 2001; Sandrini et al., 2005), but some groups also reported that N/OFQ causes hyperalgesia and analgesia (Meunier et al., 1995; Mogil and Paternak, 2001; Reinscheid et al., 1995; Suaudeau et al., 1998). Recent studies (Heinricher, 2005; Neubert et al., 2004) have shown that the actual effect of N/OFQ depends on which neurons have been activated, off-cell or on-cell (pain-inhibiting neuron and pain-facilitating) when the peptide is given. OFQ inhibited the function of both cells (Heinricher et al., 1997; Mogil and Paternak, 2001). That means N/OFQ may block antinociception, induce hyperalgesia, or produce antinociception, depending on which neurons are activated that time.

The data reported here support the idea that N/OFQ is an endogenous anti-opioid peptide in the brain of rats in the CWT test. This test measures a different type of pain than was reported in previous studies. Thus, it extends the anti-opioid concept to yet another system and generalizes the phenomenon.

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6. References

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Figure 1.

N/OFQ blockade of antinociception induced by s.c. injection of morphine (8 mg/kg). $P < 0.01$ for morphine + saline group vs morphine + N/OFQ group. $P > 0.05$ for saline + saline group vs saline + N/OFQ group. $N = 6-7$ per group. Each point represents the mean + S.E.M.

Figure 2. N/OFQ blockade of antinociception induced by s.c. injection of spiradoline (80 mg/kg). $P < 0.01$ for spiradoline + saline group vs spiradoline+N/OFQ group. $P > 0.05$ for saline + saline group vs saline + N/OFQ group. $N = 5-7$ per group. Each point represents the mean + S.E.M.

Figure 3.
N/OFQ blockade of antinociception induced by i.c.v. injection of mu opioid receptor agonist PL017 (2 μ g/5 μ l). $P < 0.01$ for PL017+saline group vs PL017+N/OFQ group. $P > 0.05$ for saline + saline group vs saline + N/OFQ group. $N = 8-10$ per group. Each point represents the mean + S.E.M.

Figure 4.

N/OFQ blockade of antinociception induced by i.c.v. injection of delta opioid receptor agonist DPDPE (50 ng/5 μ l). $P < 0.01$ for DPDPE+saline group vs DPDPE + N/OFQ group. $P > 0.05$ for saline + saline group vs saline + N/OFQ group. $N = 9-12$ per group. Each point represents the mean $\pm$ S.E.M.

Figure 5.

N/OFQ blockade of antinociception induced by i.c.v. injection of kappa opioid receptor agonist dynorphin (10 nmol/5 µl). $P < 0.01$ for dynorphin + saline group vs dynorphin + N/OFQ group. $P > 0.05$ for saline + saline group vs saline + N/OFQ group. $N = 8-9$ per group. Each point represents the mean + S.E.M.