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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

Xiaohong Chen *, Ellen B. Geller, Martin W. Adler

Center for Substance Abuse Research, Temple University School of Medicine, 3400 N. Broad Street, Philadelphia, PA 19140, United States

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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 study was 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 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: Nociceptin/orphanin FQ; 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 ORL_1 (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,b). NOP receptors are found throughout almost all areas of the central nervous system, including spinal cord dorsal horn, nucleus raphe magnus, and the periaqueductal gray (Neal et al., 1999a,b). 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. (1996) found

* Corresponding author. Tel.: +1 215 707 5305; fax: +1 215 707 1904.
E-mail address: chenyang@temple.edu (X. Chen).

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that N/OFQ (1 or 10 μg) 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,b) 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±1 °C and approximately 50±5% 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)] × 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 μl followed by 3-μl 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

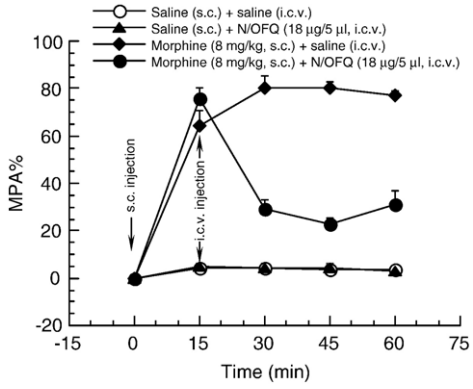

Fig. 1. N/OFQ blockade of antinociception induced by s.c. injection of morphine (8 mg/kg). $P<0.01$ for morphine+saline group vs morphine+N/OFQ group. $P>0.05$ for saline+saline group vs saline+N/OFQ group. $N=6–7$ per group. Each point represents the mean+S.E.M.