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Short communication

Antisense oligonucleotides to human SQA-neuropeptide FF decrease morphine tolerance and dependence in mice

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Abstract

Neuropeptide FF (Phe–Leu–Phe–Gln–Pro–Gln–Arg–Phe–NH_2) is able to modulate opioid analgesia. Intracerebroventricular treatment for 5 days with antisense-oligodeoxynucleotides complementary to the sequence of human SQA-neuropeptide FF (Ser–Gln–Ala–Phe–Leu–Phe–Gln–Pro–Gln–Arg–Phe–NH_2) precursor gene or by mismatch-oligodeoxynucleotides did not change the antinociceptive activity of morphine in the mouse tail flick test. In contrast, antisense- but not mismatch-oligodeoxynucleotides attenuated significantly the tolerance to the analgesic activity of morphine and the withdrawal syndrome precipitated by naloxone in morphine-treated mice. These treatments with oligodeoxynucleotides did not modify neuropeptide FF-immunoreactivity content in whole brain but repeated injections of an agonist of neuropeptide FF receptors increased the intensity of morphine tolerance. These results demonstrate the important role of neuropeptide FF in opioid pharmacodependence.

Keywords: Neuropeptide FF; Anti-opioid; Opioid tolerance; Opioid dependence; Antisense oligonucleotide; Morphine

1. Introduction

Neuropeptide FF (Phe–Leu–Phe–Gln–Pro–Gln–Arg–Phe–NH_2) is an amidated peptide detected in several animal species (Panula et al., 1996) and possessing anti-opioid activity since i.c.v. injections of neuropeptide FF reverse morphine-induced analgesia (Roumy and Zajac, 1998). However, neuropeptide FF also exhibits pro-opioid effects because neuropeptide FF and neuropeptide FF analogs inhibit intestinal transit in mice (Gicquel et al., 1993) and produce analgesia after intrathecal administration in rats (Gouardères et al., 1996). Results of several experiments suggest that neuropeptide FF could participate in morphine tolerance and dependence, and in fact this peptide induces some signs of the withdrawal syndrome in morphine-dependent rats (Malin et al., 1990a). Similarly, central immunoneutralization of neuropeptide FF reversed morphine tolerance (Lake et al., 1991) and decreased the score of abstinence signs precipitated by naloxone (Malin et al., 1990b). These features suggest that neuropeptide FF acts as a neuromodulator of opioid systems.

Very recently, one gene encoding the precursor of neuropeptide FF was identified in the human (Perry et al., 1997), revealing two novel neuropeptides, human neuropeptide AF (Ala–Gly–Glu–Gly–Leu–Asn–Ser–Gln–Phe–Trp–Ser–Leu–Ala–Ala–Pro–Gln–Arg–Phe–NH_2) and SQA-neuropeptide FF (Ser–Gln–Ala–Phe–Leu–Phe–Gln–Pro–Gln–Arg–Phe–NH_2). The sequences of human SQA-neuropeptide FF and bovine neuropeptide FF are identical except for a three-amino acid N-terminal extension in the human peptide (Ser–Gln–Ala). The human peptide was described as inactive on the monosynaptic component of the ventral root reflex response of rat spinal cord (Perry et al., 1997) but biochemical, cellular and pharmacological activities of SQA-neuropeptide FF are identical to those of neuropeptide FF in mice (Gelot et al., 1998). These findings suggest that neuropeptide FF could also play a role in the human but, in the absence of antagonists of the neuropeptide FF receptor, its physiological functions remain difficult to elucidate. The antisense strategy could therefore represent an interesting pharmacological tool (for review see Wahlestedt, 1994; Gold, 1996; Weiss et al., 1997) and the blockade of mRNA encoding for the neuropeptide FF precursor offers the possibility to investigate the functional role of neu-

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ropeptide FF. Our results, obtained with an antisense strategy in mice and with chronic injections of a neuropeptide FF receptor agonist, provide evidence that neuropeptide FF participates in the development of morphine tolerance and dependence.

2. Materials and methods

2.1. Chemicals

Morphine hydrochloride was obtained from Francopia, naloxone from Sigma, [D-Tyr^1,(NMe)Phe^3]neuropeptide FF was synthesized as previously described (Gicquel et al., 1994). These compounds were dissolved in saline before injection.

Phosphorothioated oligodeoxynucleotides (18 mers) were purchased from Isoprim (Toulouse, France). Control oligodeoxynucleotides were mismatch-oligodeoxynucleotides in which four nucleotides were switched without altering the remaining sequence. An equimolar mixture of two antisense- or two mismatch-oligodeoxynucleotides was made, to yield a final concentration in sterile water of 2×10^{-4} M. These two mixtures were injected (1 nmol) into the lateral cerebral ventricle at 0900 for 5 days. Sequences were as follow: oligo 5′-TAACAGCAGCAG-CACCAG-3′, complementary to nucleotides 28–45 of the coding region, (antisense) and oligo 5′-AAACACCAG-CAGCTCGAG-3′ for the corresponding mismatch-oligodeoxynucleotide; oligo 5′-AACCTCTGGG-GCTGAAAC-3′, complementary to nucleotides 209–227 of the coding region (antisense) and oligo 5′-AAGCTCTG-GGGCAGATAG-3′ (mismatch). The sequences were checked to ensure that no cross-hybridization could occur with known mRNA (Fasta, Gen Bank). The complete sequence of the SQA-neuropeptide FF precursor was registered as Gen Bank no. AF005271.

2.2. Animals and antinociceptive testing

The animals were tested in accordance with the ethical guidelines of the International Association for the Study of Pain. Male Swiss mice (20–25 g, C.E. Depré, France) were maintained at 21 ± 0.5°C on a 12:12 h dark/light cycle with free access to food and water. Groups of 10 mice were used for each dose.

Nociceptive responses were assessed in the tail-flick test with a cut-off time set to 8 s. Baseline tail withdrawal latencies were measured prior to chronic treatments and 5 and 10 min before s.c. injection of morphine. The test was performed 15, 30, 45 and 60 min after s.c. injection of morphine, i.e., 7 h after the last i.c.v. administration of vehicle or oligodeoxynucleotides. The maximum percentage effect (% MPE) was calculated as: $100 \times [(\text{post-injection latency} - \text{baseline latency})/(8 - \text{baseline latency})]$.

2.3. Tolerance and dependence studies

After i.c.v. injections of antisense- or mismatch-oligodeoxynucleotides or sterile water (control) for 2 days, morphine was concurrently injected i.p. as follows: 50 mg kg^{-1} at 1000 and 1600 h on day 3; 50 mg kg^{-1} at 1000 and 1400 h, and 100 mg kg^{-1} at 1800 h on day 4; 100 mg kg^{-1} at 0900 h on day 5.

Withdrawal signs were precipitated by injecting 5 mg kg^{-1} i.p. naloxone, 5 h after the last i.p. morphine administration. Each mouse was placed in a Plexiglass cylinder (30 cm high, 18 cm diameter) and the number of vertical jumps was counted over 30 min.

For chronic i.c.v. treatments with [D-Tyr^1,(NMe)-Phe^3]neuropeptide FF, the mice were injected at 1000 h for 7 days with saline or 2.2 nmol [D-Tyr^1,(NMe)-Phe^3]neuropeptide FF; 5.5 nmol morphine or 5.5 nmol morphine plus 2.2 nmol [D-Tyr^1,(NMe)Phe^3]neuropeptide FF. The antinociceptive activity of i.c.v. morphine was evaluated 24 h after the last injection.

2.4. Neuropeptide FF-immunoreactivity content in brain

Brains dissections and tissue extractions were performed according to Allard et al. (1991). All dilutions were performed in 0.1 M phosphate buffer (pH 7.4) containing 0.05 M NaCl and 0.1% bovine serum albumin. A volume of 100 μl of diluted standard peptide or of a tissue extract was incubated overnight at 4°C with 100 μl of a neuropeptide FF antiserum (final dilution 1:150 000) and 100 μl of 40 pM [^{125}I][D-Tyr^1,(NMe)Phe^3]neuropeptide FF. Non-specific binding was determined in the presence of 0.3 μM neuropeptide FF. Free and bound radioligand were separated by adding a charcoal suspension. The samples were centrifuged and bound radioactivity was quantified by counting 0.4 ml of the supernatant in a Packard Cobra auto-gamma counter.

The results for each group were expressed as the median value of the neuropeptide FF immunoreactivity content (minimum value − maximum value) in fmol/mg tissue and compared by χ^2 tests.

3. Results

3.1. Oligodeoxynucleotides and morphine tolerance

The analgesic potency of s.c. morphine in naive mice was unaffected by treatments with antisense- or mismatch-oligodeoxynucleotides (Table 1 and Fig. 1). After 3 days of morphine administration, the dose–response curves for morphine were shifted to the right, revealing opioid tolerance (Fig. 1). Comparison of the morphine ED_{50} values showed that mice injected with antisense-oligodeoxynucleotides were significantly more sensitive to morphine than were mismatch-oligodeoxynucleotide-

Table 1
Morphine tolerance and dependence in mice treated with antisense oligonucleotides to human SQA-neuropeptide FF precursor

	I.c.v. treatment		
	Control	Antisense	Mismatch
(A) s.c. morphine ED_{50} (mg kg^{-1})			
Naive mice	1.53 ± 0.28	2.65 ± 0.21	2.35 ± 0.79
Tolerant mice	12.15 ± 0.10	9.12 ± 0.54	15.12 ± 0.26
ED_{50} ratio	7.9	3.4	6.4
(B) Number of jumps			
	213.0 ± 44.5	$91.4 \pm 37.7^{a,b}$	193.0 ± 42.4

(A) Tolerance. Results are expressed as antinociceptive ED_{50} values ± S.E.M., calculated 30 min after s.c. administration (peak effect) of morphine.
(B) Dependence. The withdrawal syndrome was precipitated by administration of 5 mg kg^{-1} i.p. naloxone and was quantified by the number of jumps over 30 min. Data are expressed as means ± S.E.M.
[a] $P < 0.05$ vs. injection of mismatch-oligodeoxynucleotides.
[b] $P < 0.05$ vs. control (unpaired Student's *t*-test).

treated mice (Table 1). The degree of tolerance, evaluated from the ED_{50} ratios between morphine-treated and morphine-naive mice (7.9 in control mice) was not modified by injections of mismatch-oligodeoxynucleotides (6.4) but was halved (3.4) in mice injected with antisense-oligodeoxynucleotides. Thus, 15 mg kg^{-1} s.c. morphine had a significant analgesic effect in antisense-oligodeoxynucleotides-treated mice but was nearly inactive in control or mismatch-oligodeoxynucleotides-treated mice (AUC values for 1 h: 2854 ± 295, antisense; 1759 ± 215 and 1771 ± 330% MPE × min for control or mismatch). These results suggest strongly that injections of antisense oligonucleotides to neuropeptide FF attenuate morphine tolerance.

3.2. Oligodeoxynucleotides and morphine dependence

A subcutaneous injection of 5 mg kg^{-1} naloxone to chronic morphine-treated mice elicited a withdrawal syndrome, as evidenced by a characteristic jumping behaviour (213 jumps/30 min). This score was significantly reduced in mice pretreated with antisense-oligodeoxynucleotides but not in mice injected with mismatch-oligodeoxynucleotides (Table 1).

3.3. Oligodeoxynucleotides and brain neuropeptide FF-immunoreactivity content

In mice treated for 5 days with antisense- and mismatch-oligodeoxynucleotides, the neuropeptide FF immunoreactivity content in total brain extracts amounted to 3.59 (1.0–175) and 1.60 (0.9–120.2) fmol/mg tissue, respectively. The distribution of values was not binomial and was characterized in both cases by the occurrence of high values not observed in mice receiving vehicle alone. A χ^2 test did not reveal significant differences between antisense-, mismatch-oligodeoxynucleotides or control groups. Chronic morphine treatment did not modify significantly the neuropeptide FF immunoreactivity content in total brain, the median values in control and morphine-treated mice being very similar, 1.53 (1.1–5.56) and 1.55 (0.75–3.88) fmol/mg tissue, respectively. Mice chronically treated with morphine and concurrently with i.c.v. injections of antisense- or mismatch-oligodeoxynucleotides did not have significantly different neuropeptide FF immunoreactivity content; 6.85 (0.8–46) and 1.74 (0.75–63) fmol/mg tissue.

3.4. Chronic injections of [D-Tyr[1],(NMe)Phe[3]]neuropeptide FF and morphine tolerance

Daily intracerebroventricular injections of morphine (5.5 nmol) for 7 days induced tolerance, since the ED_{50} value (and 95% Confidence Limits) was 6.6-fold higher in morphine-treated than in naive mice; 10.5 (6.3–17.6) vs. 1.6 (0.9–3.0) nmol. Seven days' injections with 2.2 nmol [D-Tyr[1],(NMe)Phe[3]]neuropeptide FF, an agonist of neuropeptide FF receptors resistant to peptidases (Gicquel et al., 1993), decreased morphine activity (ED_{50} = 7.1 (2.4–20.3) nmol. Chronic co-injections of morphine and [D-Tyr[1],(NMe)Phe[3]]neuropeptide FF shifted the morphine ED_{50} value to 146.2 (56.5–378.6) nmol. The degree of opioid tolerance expressed as the ED_{50} ratio (chronic morphine/naive) was three-fold higher in [D-Tyr[1],(NMe)Phe[3]]neuropeptide FF-treated mice, suggesting that chronic injections of a neuropeptide FF receptor agonist could increase the level of opioid tolerance.

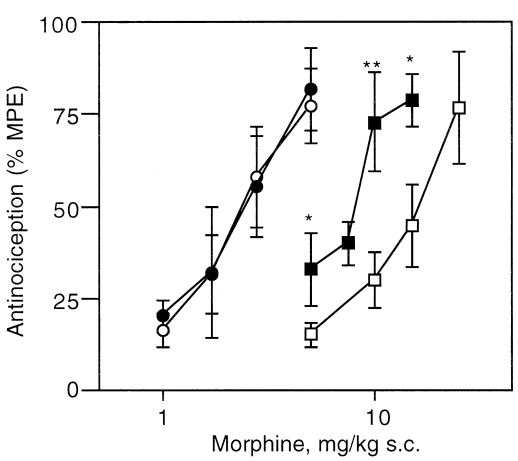

Fig. 1. Antinociceptive activity of s.c. morphine in naive and morphine-treated mice. Morphine was injected 7 h after the last i.c.v. administration of antisense (antisense-oligodeoxynucleotides, filled symbols) or mismatch (mismatch-oligodeoxynucleotides, open symbols) oligonucleotides to human neuropeptide FF precursor in naive (circle) or morphine-treated (square) mice. The antinociceptive activity of morphine (% MPE) in the tail flick test was calculated at the peak effect of morphine (30 min after injection). ** $P < 0.01$, * $P < 0.05$ vs. mismatch-oligodeoxynucleotide-treated mice (unpaired Student's *t*-test).

4. Discussion

In a classical anti-opioid model of pharmacodependence, repeated injections of opioids induce a compensatory increase of anti-opioid peptide release which attenuates the pharmacological effects of opioids. The cessation of opioid administration leads to an excess of anti-opioids and gives rise to a number of signs of the withdrawal syndrome. Pharmacological data indicate that neuropeptide FF exhibits anti-opioid properties, and antisense oligonucleotides to human neuropeptide FF precursor were used in the present study in order to demonstrate directly the involvement of neuropeptide FF in the development of opioid pharmacodependence in mice.

Inhibition of the actions of endogenous neuropeptide FF by antisense-oligonucleotides (this study) or by an antiserum (Lake et al., 1991) did not modify the acute antinociceptive effect of morphine, suggesting that the level of endogenous neuropeptide FF required to obtain an anti-opioid effect is probably reached only at the end of chronic morphine treatment. Repeated injections of antisense to human neuropeptide FF significantly reduced the intensity of morphine tolerance and dependence in mice. This result suggests a high homology of sequences between rodent and human precursors but the mechanism of this effect remains unclear because the neuropeptide FF immunoreactivity content was not significantly modified in whole brain after antisense-oligodeoxynucleotide injections. In rats, the neuropeptide FF-like immunoreactivity increased in the CSF after chronic administration of morphine (Malin et al., 1990b) although, in an other study, a significant decrease of the neuropeptide FF immunoreactivity content, 1 h after morphine pellet implantation, was followed by a drastic increase between 3 and 6 h (Stinus et al., 1995). In the present study, discrete and localized changes could have been masked in total brain extracts. Detailed analysis in different brain regions specifically involved in opioid tolerance and dependence could help to detect significant changes in neuropeptide FF release after antisense-oligodeoxynucleotide treatment.

According to the anti-opioid model, the blockade of neuropeptide FF expression should diminish opioid tolerance and, conversely, over-stimulation of neuropeptide FF receptors should increase the intensity of opioid tolerance and dependence. Thus, the observation that intracerebroventricular co-treatment with morphine and a neuropeptide FF receptor agonist augments the intensity of opioid tolerance in mice further supports the validity of the anti-opioid model of tolerance.

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