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Involvement of endogenous Orphanin FQ in electroacupuncture-induced analgesia

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RECENT studies suggest that the novel opioid peptide orphanin FQ (OFQ) is involved in pain modulation. We found that intracerebroventricular (i.c.v.) administration of OFQ in the rat produced a dose-dependent antagonism of the analgesia induced by 100 Hz electroacupuncture (EA) stimulation as measured in the radiant heat tail-flick assay. Antisense oligonucleotides injected i.c.v. potentiated EA analgesia, presumably by interfering with the expression of the OFQ receptor in brain. These results suggest that endogenous OFQ exerts a tonic antagonistic effect on EA-induced analgesia. No such antagonism was observed when OFQ was injected intrathecally (i.t.). Rather, it appears that spinal OFQ produced a marked analgesic effect and enhanced EA-induced analgesia. These findings are consistent with the experimental results obtained in rats where morphine-induced analgesia is antagonized by i.c.v. OFQ and potentiated by i.t. OFQ.

Key words: Antisense oligodeoxynucleotide; Orphanin FQ; LC132; Nociceptin; Opiate; Electroacupuncture-induced analgesia; Pain inhibition

Introduction

Despite the large degree of structural and sequence overlap between the well characterized opioid peptides met- and leu-enkephalin, dynorphin, and β-endorphin, preliminary reports have suggested that the newest member of the opioid peptide family, orphanin FQ (OFQ)[1] or nociceptin,[2] is not an analgesic. Although the initial reports suggested that OFQ/nociceptin may even produce hyperalgesia,[1,2] subsequent studies have demonstrated that the apparent hyperalgesia reported was in fact a reversal of the stress-induced analgesia (SIA) that developed as a consequence of the intracerebroventricular (i.c.v.) route of drug administration,[3] and that OFQ in fact, functionally antagonizes morphine analgesia[3,4] as well as analgesia resulting from activation of μ, δ or κ opioid receptors.[5]

Our previous studies demonstrated that endogenous opioid peptides play an important role in the mediation of electroacupuncture (EA)-induced analgesia.[6,7] In the present study, we attempted to characterize the effect of synthetic OFQ on EA analgesia, and to explore the role of endogenous OFQ on EA analgesia by using antisense oligodeoxynucleotides designed to block the expression of the gene encoding the OFQ receptor (also called LC132/ORL1).

Materials and Methods

Adult female Wistar rats weighing 220–280 g were provided by the Animal Center, Beijing Medical University. Between nine and 17 animals were used in each group. The implantation of both i.c.v. cannulae and intrathecal (i.t.) catheters was performed under chlorohydrate (10%) anesthesia (0.3 ml 100 g^{-1} b.wt.). For i.c.v. cannulae, stainless steel tubing (0.8 mm o.d.) was fixed on the skull at stereotaxic coordinates A 5.4, L 1.5, H 3.0 mm, according to the system of Pellegrino *et al.*[8] Analgesia experiments involving i.c.v. injection started 3–4 days after the operation. Intrathecal catheterization was performed according to the method of Yaksh and Rudy.[9] PE-10 tubing 13 cm in length was inserted through the incised atlanto-occipital membrane and dura into the subarachnoid space for 7.5 cm, to reach the upper border of the lumbar enlargement. Injections were started one day post surgery. For both types of central injections, the volume administered was 10 μl, delivered over 10 s.

Orphanin FQ (complete 17 amino acid sequence) purchased from Phoenix Pharmaceuticals, Inc., Mountainview, CA USA, was dissolved in sterile NS and administered in a range of doses from 0.1 to 10.0 nmol. An antisense oligodeoxynucleotide complimentary to the OFQ receptor, and a missense

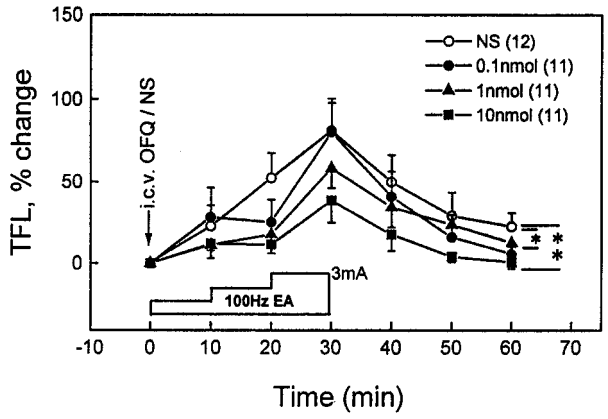

FIG. 1. Effect of i.c.v. OFQ on 100Hz EA analgesia in rats. Shown are the time–effect curves of 100 Hz EA analgesia in 60 min, which is dose-dependently reversed by increasing doses of OFQ. Numbers in parentheses show the number of rats in each group. Each point represents the mean ± s.e. $*p < 0.05$, $**$ $p < 0.01$, compared with saline control group, tested by ANOVA followed by the Newman-Keuls test.

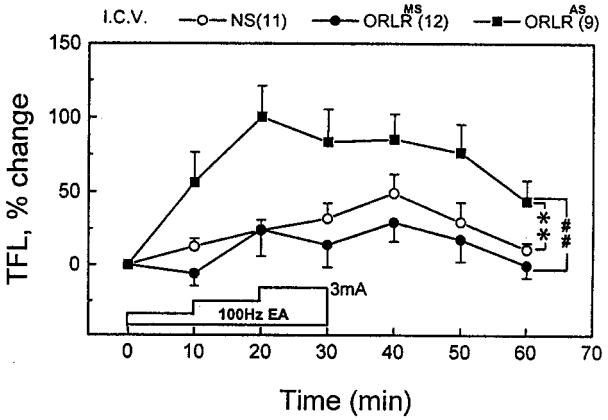

FIG. 2. Effect of 100 Hz EA after i.c.v. treatment with saline, missense oligodeoxynucleotide (ORLR ^{MS}) and antisense oligodeoxynucleotide (ORLR ^{AS}) complimentary to the OFQ receptor (opioid receptor-like receptor, ORLR). Numbers in parentheses show the number of rats in each group. Each point represents the mean ± s.e. $**p < 0.01$, compared with saline group, $^{\#\#}p < 0.01$, compared with missense oligonucleotide group, tested by ANOVA followed by the Newman-Keuls test.

oligodeoxynucleotide (mismatched control) were synthesized by GIBCO/BRL, Bethesda, MD USA. The antisense oligonucleotide was complimentary to bases 9–25 of the translated region of the rat OFQ receptor mRNA[10–12] and had the sequence 5′-ATGGAGCAGGAAAGAGG-3′. The missense oligonucleotide of the same length and base composition as the antisense oligonucleotide had the random sequence, 5′-ATCGAGGAGGAGAGAGA-3′. The oligonucleotides were dissolved in sterile normal saline at a final concentration of 2 mg ml^{-1}. The rats received i.c.v. injections containing 20 mg oligonucleotide or normal saline on days 1, 3 and 5 and were tested on day 6.

Experiments were performed at room temperature (23 ± 1°C). Nociception was tested by radiant heat-induced tail flick latency.[13] Rats were lightly restrained in a plastic holder with their hind legs and tail extending and light from a 12.5 W projection bulb was focused on the lower third of the tail. The latency to tail flick (TFL) response was recorded to the nearest 0.1 s by an experienced observer, blind to drug condition. Values from the first three measurements, with an interval of 5 min, were averaged as the basal TFL, usually within the range of 4–6 s. TFL obtained in subsequent measurements were expressed as percentage change from the basal level, with a cut-off limit of +150% to avoid tissue damage.

Electroacupuncture (EA) stimulation was administered via stainless steel needles inserted 5 mm into two sites on the hind legs: one in the Zusanli point near the knee joint (ST36, 5 mm lateral to the anterior tubercle of tibia) and the other at the Sanyinjiao point near ankle joint (SP6, at the level of the superior border of the medial melleolus, between the posterior border of the tibia and the anterior border of the Achilles tendon). The two needles were connected to a WQ-8D electronic stimulator (Beijing Aviation Institute, Beijing, China) which delivered square wave pulses of 0.3 ms pulse width, with constant current output adjustable in the range 0–3 mA and with a frequency of 100 Hz. The intensity was set at 1 mA and increased stepwise to 2 mA and then 3 mA, each of which lasted for 10 min. Animals were observed for 60 min (TFL assessed every 10 min).

Data were expressed as the mean ± s.e.m. for each group. Statistical analysis of difference between groups was assessed with a two-way analysis of variance (ANOVA) followed by Newman-Keuls test. $p < 0.05$ was taken as the significant level of difference.

Results

The effect of OFQ on EA analgesia is shown in figure 1. Rats were randomly divided into four groups, individually assessed for their baseline nociceptive sensitivity and then given i.c.v. injections (0.1, 1 or 10 nmol OFQ or normal saline). They were then immediately given 100 Hz EA for 30 min during which TFL was determined at 10, 20 and 30 min. TFL was also assessed for an additional 30 min following the cessation of EA stimulation (again, at 10 min intervals). EA stimulation increased TFL in the control (saline-treated) group by 81 ± 16.8%. OFQ (1.0 and 10.0 nmol, but not 0.1 nmol) significantly reversed the EA analgesia resulting from 30 min 100 Hz stimulation ($p < 0.05$ and $p < 0.01$, respectively). OFQ attenuated the EA-induced analgesia rather than altering nociception directly, as i.c.v. injection of OFQ alone did not affect TFL at any of

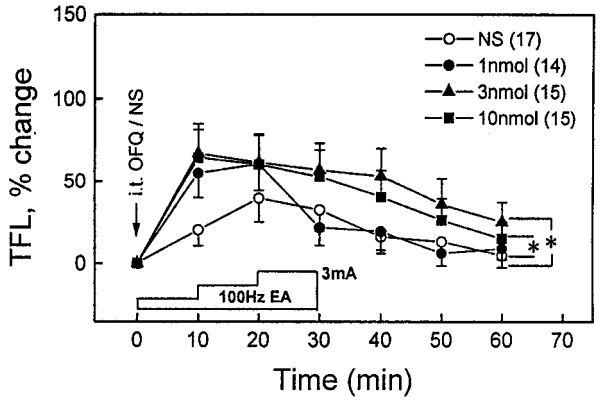

FIG. 3. Effect of i.t. OFQ on 100 Hz EA analgesia in rats over 60-min time courses. Numbers in parentheses show the number of rats in each group. Each point represents the mean ± s.e. $*p < 0.05$, compared with saline group, tested by ANOVA followed by the Newman-Keuls test.

the above doses as determined in radiant-heat test in rats (data not shown).

The antisense oligodeoxynucleotide complimentary to the OFQ receptor potentiated EA analgesia (Fig. 2). Rats were randomly assigned to repeated injections of saline, antisense or missense oligodeoxynucleotide. While there was no significant difference between the saline- and missense oligodeoxynucleotide-treated groups, animals receiving antisense oligonucleotide showed greatly enhanced EA analgesia compared with both of the other two groups ($p < 0.01$). In contrast, i.t. administration of OFQ did not antagonize EA analgesia (Fig. 3). Unlike i.c.v. OFQ (1.0 or 10.0 nmol), which significantly attenuated EA analgesia, i.t. OFQ slightly potentiated the analgesia inuced by 100 Hz EA. EA *per se* produced a mild analgesia, especially at 20 min and 30 min time points. The enhancement by i.t. OFQ was most prominent in the first 10 min of EA and after the termination of EA.

To explore further the effect of i.t. OFQ on nociception, four additional groups of rats were given either saline or 1.0, 3.0 or 10.0 nmol OFQ and assessed for TFL every 10 min for a 60 min period. OFQ produced a dose-dependent increase in TFL, which lasted for only 10–20 min (Table 1). It is obvious that when i.t. OFQ is given in combination with EA, the increase of TFL in the first 10 min is accounted for mainly by OFQ, whereas the analgesia seen at 30–50 min is an additive or mild synergistic effect between EA and OFQ.

Discussion

It has been clearly shown that i.c.v. injection of OFQ produces a dose-dependent antagonism of morphine-induced analgesia.[3,4,14] OFQ also blocks stress-induced opioid-mediated analgesia.[3] It seems likely, therefore, that OFQ also modulates endogenous opioid activity. One of the methods employed to activate the release of endogenous opioid peptide is the use of identified electrical stimulation of peripheral sites, referred to as transcutaneous electrical nerve stimulation (TENS) or electroacupuncture (EA) stimulation. The mechanisms whereby EA induces the release of opioid peptides have, to a large extent, been elucidated.[15] For example, 100 Hz EA is known to increase the release of dynorphin in the spinal cord to produce analgesia.[16,17] To explore the influence of OFQ on 100 Hz EA analgesia we administered OFQ either i.c.v. or i.t. immediately prior to 100 Hz stimulation. I.c.v. injection of synthetic OFQ produced a clear-cut, dose-dependent antagonism of 100 Hz EA-induced analgesia, implicating an interaction between exogenous OFQ and endogenous opioids, especially dynorphin. This is consistent with our pervious results that suggested that OFQ blocks the analgesia produced by the κ agonist, U50,488H.[5]

We cannot currently measure endogenously released OFQ during EA stimulation, nor is a specific antagonist against OFQ receptor available. One method however, of studying the function of endogenous OFQ is to block the synthesis of OFQ receptor using antisense oligonucleotides. To achieve this, three successive i.c.v. injections of antisense oligodeoxynucleotides were given over 5 day period. This time-course was based on the reported turnover rate of opioid receptor proteins being 3–5 days.[18] A missense oligonucleotide was used as control. Consistent with our hypothesis that OFQ acts as a functional anti-opioid in assays of nociception, the antisense but not the missense oligonucleotide produced a marked augmentation of the EA analgesia. This result implies that endogenously released

Table 1. Effect of OFQ (i.t.) on basal TFL

OFQ (nmol)	*n*	Percentage change of TFL after i.t. injection of OFQ					
		10 min	20 min	30 min	40 min	50 min	60 min
0	10	7.6 ± 2.8	2.4 ± 3.3	9.4 ± 7.6	5.0 ± 4.9	5.6 ± 3.9	7.1 ± 5.0
1	9	15.6 ± 4.0	20.1 ± 6.6	10.7 ± 5.4	4.8 ± 3.2	20.1 ± 4.5	12.0 ± 4.1
3*	10	63.6 ± 20.1*	36.4 ± 16.3	21.0 ± 15.2	22.4 ± 14.6	4.2 ± 1.8	7.3 ± 2.2
10**	13	82.6 ± 17.9**	52.7 ± 15.3	31.2 ± 13.9	34.7 ± 15.1	35.0 ± 14.6	24.7 ± 10.8

Results are expressed as mean ± s.e. $*p < 0.05$, $**p < 0.01$, compared with saline-treated group.

OFQ plays an antagonistic role in EA induced analgesia via activation of the OFQ receptor.

The likelihood that the effect of i.c.v. OFQ occurs via diffusion of i.c.v.-administered substrates into the spinal cord seems to be low.[19] In mice, antagonism of opiate analgesia by OFQ appears to be limited to supraspinal sites.[14] Additional evidence supporting this conclusion is that OFQ administered directly to the spinal cord has no antagonistic effect on EA analgesia (Fig. 3). The mechanisms whereby brain OFQ exerts an antagonistic effect on 100 Hz EA-induced analgesia, which is mediated primarily by spinally released dynorphin,[16,17] are yet to be elucidated. The possibility that OFQ mediates this effect via a descending pain facilitatory system deserves serious consideration.

In contrast to the robust effect of OFQ on supraspinal opioid processes, the effect of spinal OFQ on 100 Hz EA was not as clear-cut or dramatic. What attracts us most is the fact that OFQ appeared to produce a marked although transient analgesia on its own as measured in the radiant heat tail-flick assay. Stanfa *et al* demonstrated recently that i.t. nociceptin (OFQ, 5, 50, 225 mg) dose-relatedly inhibited the C-fiber evoked wind-up and post-discharge of dorsal horn neurons in the rat *in vivo*, which suggested an antinociceptive role of 'nociceptin' in the spinal cord.[20] Further studies are needed to clarify the role played by spinally released endogenous OFQ in the modulation of nociceptive sensitivity.

Conclusion

The novel neuropeptide Orphanin FQ, which is the endogenous agonist for the opioid-like receptor, plays different roles in the brain and spinal cord of the rat. Orphanin FQ in the brain displayed a marked antagonism of 100 Hz electroacupuncture (EA)-induced analgesia which is believed to be mediated by endogenous dynorphin. In contrast, OFQ in the spinal cord produced a mild augmentation of EA analgesia. These results are consistent with the interpretation that the interaction between endogenously released OFQ and opioid peptides is similar to, if not identical with, those interactions that occur between i.c.v. administered OFQ and morphine.

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General Summary
Orphanin FQ (OFQ) is a recently discovered endogenous opioid-like peptide. Unlike the opioid peptides OFQ appears to act as a functional antiopioid: in assays of nociception (pain sensitivity) opioids produce an analgesia that OFQ reverses. Electroacupuncture (EA) has been shown to produce analgesia by stimulating the release of endogenous opioid peptides. In the present study OFQ injected into the brains of rats caused a dose-dependent antagonism of the analgesia induced by 100 Hz EA stimulation as measured in the radiant heat tail-flick assay. In lieu of specific OFQ antagonists antisense oligonucleotides were developed and injected into rat brains resulting in the potentiation of EA analgesia, presumably by interfering with the expression of the OFQ receptor. Our results suggest that endogenous OFQ in brain exerts a tonic antagonistic effect on EA-induced analgesia. Given its anti-opioid actions, OFQ may be playing an important role in the development of opiate tolerance, dependence and addiction.