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Functional studies using antibodies against orphanin FQ/nociceptin

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Abstract

Orphanin FQ/nociceptin (OFQ) is a recently discovered endogenous ligand for the novel opioid receptor-like receptor (ORL-1). There are numerous reports in the literature demonstrating paradoxical effects of exogenous OFQ on pain modulation. For example, OFQ produces a pronociceptive effect in the brain and an analgesic effect in the spinal cord. In order to better understand the physiological actions of OFQ, the present study focused on the pain-modulatory effect of endogenously released OFQ measured using antibody microinjection techniques. We found that electroacupuncture analgesia (EA) was increased by intracerebroventricular (i.c.v.) injection of an OFQ-antibody and decreased following intrathecal injection. Furthermore, i.c.v. OFQ-antibody partially reversed tolerance to both chronic morphine and chronic EA. These data suggest that endogenously released OFQ plays an important role in pain modulation, where pain sensitivity in the brain and spinal cord is increased and decreased, respectively.

Keywords: Orphanin FQ; Nociceptin; Antibody; Pain; Tolerance

1. Introduction

It has been suggested that Orphanin FQ/nociceptin (OFQ) represents the fourth endogenous opioid peptide and joins the ranks of the well-described enkephalins, endorphins, and dynorphins. The description of the chemical structure of OFQ was immediately followed by a flurry of biochemical and physiological studies. One of the most interesting questions was whether OFQ possessed analgesic properties similar to other opioid peptides.

The actions of OFQ on pain modulation have been shown to depend on the site of administration, and consensus opinion is that OFQ exhibits pronociceptive actions in the brain (hence the name nociceptin) and antinociceptive actions in the spinal cord [4,8–10,12–15,19]. These data were obtained using conventional pharmacological methodology where exogenous peptide was administered directly into the central nervous system (CNS). Using this paradigm, it is not possible to differentiate the physiological from pharmacological effects of the applied peptide.

More rational approaches for the study of an endogenously released neuropeptide are based around examining the effects of a functional deficit. This functional deficit can be produced by blockade of peptide biosynthesis, neutralization of the released peptide, and/or occupation of its specific receptor.

The current understanding of the metabolic processing of neuropepetides is far less clear than those for classic neurotransmitters; it is therefore premature to design a specific inhibitor targeting the enzymatic processing of an identified peptide. While the opioid receptor-like receptor 1 (ORL1)/LC132 has been demonstrated to be the specific receptor for OFQ, there is still a relative paucity of specific antagonists for this system. In 1998, Guerrini et al. [6] reported that [Phe[1] 89 (CH_2-NH) Gly[2]]NC(1–13)]NH_2 acted as a specific antagonist for OFQ receptor found in a range of peripheral tissues. However, due to the variable agonist and antagonist activity of this peptide in a range of preparations, its usefulness is questionable [1–3,5,17,18].

2. Rationale and methodological considerations for the use of OFQ antibodies

The administration of specific antibodies against OFQ to discrete brain sites, thereby neutralizing endogenous pep-

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tide released from nerve terminals, represents an additional approach to study the physiological function of OFQ. More specifically using antibody microinjection techniques, it is possible to produce selective OFQ inactivation. This approach will provide a unique opportunity to differentiate OFQ from other closely related peptides and represents a major advantage of antibody approaches over receptor antagonists, which can not differentiate the effects of several agonists active at the same receptor. A good example is the opioid antagonist naloxone, which blocked the effects of both met-enkaphalin (MEK) and met-enkephalin-Arg6-Phe7 (MEAP) simultaneously, whereas antibodies against MEAP selectively abolished the effect of MEAP, leaving that of MEK intact [7].

In order to control for the nonspecific actions of a polyclonal antiserum, the following precautions were taken: 1) Rather than using whole antiserum the IgG fraction obtained by affinity chromatography on a protein A sepharose column was used; 2) The IgG fraction obtained from normal rabbit serum was used as a control; 3) "Dose" response curves were constructed using a series of IgG dilutions rather than using a fixed concentration; 4) A fixed volume of 10 μl was used for both intracerebroventricular (i.c.v.) and intrathecal (i.t.) injections; and 5) An OFQ antiserum with virtually no cross-reactivity to β-endorphin, dynorphin1–17, or OFQ1–7 was used [11].

3. Effects of OFQ antibody on morphine and EA analgesia

Our earlier work indicated that i.c.v. OFQ antagonized morphine and electroacupuncture (EA) analgesia, while i.t. OFQ increased morphine and EA analgesia [14,15]. These bidirectional modulatory actions of OFQ on morphine and EA analgesia have since been supported by others [4,8–10, 12,13,19].

We have further studied this bidirectional modulation by injecting an OFQ antibody into the cerebral ventricle and spinal subarachnoid space. Specifically, we asked the question, could i.c.v. injection of an OFQ-antibody increase and i.t. injection decrease EA analgesia? EA analgesia was produced by stimulating acupuncture points on the hind leg of the rat at a frequency of 100 Hz. Radiant heat-induced tail flick latency (TFL) was used as an index of nociception. Rats were classified as either high responders (HR) or low responders (LR) based on the analgesic effects induced by EA. LRs could be converted into HRs by i.c.v. microinjection of OFQ antibodies (IgG fraction-OFQ-Ab) at both 1:1 and 1:10 but not 1:100 dilutions (Fig. 1). HRs could be converted to LRs by i.t. injection of OFQ-Ab at 1:1 and 1:10 but not 1:100 dilutions (Fig. 2) [16]. The findings that i.c.v. injection of OFQ antibody enhanced while i.t. injection decreased EA analgesia agree with data obtained from exogenously administered peptide [15]. We suggest that this immunologic approach is both practical and effective for the study of endogenously released peptides when conventional antagonists are not readily available.

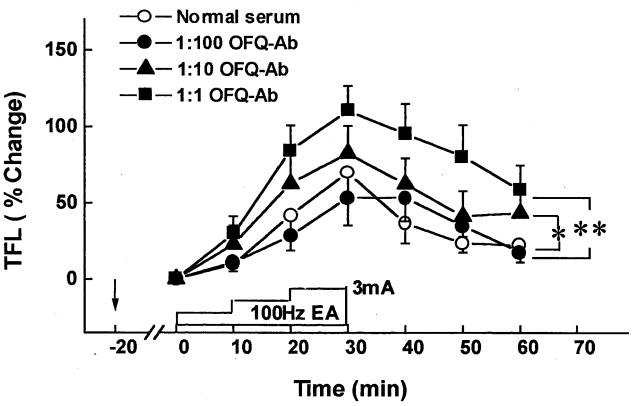

Fig. 1. OFQ antibody (OFQ-Ab) dose-dependently augmented 100 Hz EA stimulation-induced analgesia in low responder rats. OFQ-Ab or normal rabbit serum IgG (normal serum) were given i.c.v. 20 min before EA stimulation. Normal Serum (n = 12), 1:100 OFQ-Ab (n = 12), 1:10 OFQ-Ab (n = 12), 1:1 OFQ-Ab (n = 11) were injected, i.c.v., as indicated by the arrow. Vertical lines represent SEM. *, $P < 0.05$; **, $P < 0.01$, compared with normal serum-treated group by ANOVA followed by Newman–Keuls post-hoc test (See ref. 16, Fig. 1).

4. Effects of OFQ antibody on morphine and EA tolerance

Considering the potent anti-opioid actions of OFQ in the brain, one can expect that brain OFQ may also play a role in the development of tolerance to morphine and EA analgesia. We have studied this further. Chronic morphine tolerance was produced in rats by repeated injection of morphine (5–60 mg/kg, s.c., three times a day) for 6 days. In

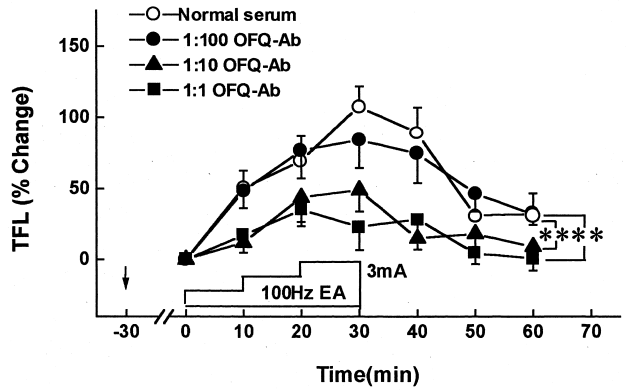

Fig. 2. OFQ-Ab dose-dependently inhibited 100 Hz EA stimulation-induced analgesia in high responder rats. OFQ-Ab or normal rabbit serum IgG (normal serum) were given i.t. 30 min before EA stimulation. Normal Serum (n = 11), 1:100 OFQ-Ab (n = 11), 1:10 OFQ-Ab (n = 10), 1:1 OFQ-Ab (n = 11) were injected, i.t., as indicated by the arrow. Vertical lines represent SEM. **, $P < 0.01$, compared with normal serum-treated group by ANOVA followed by Newman–Keuls post-hoc test (See ref. 16, Fig. 2).

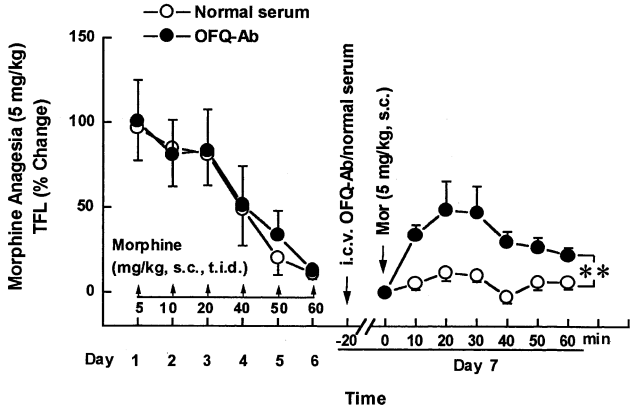

Fig. 3. Reversal of chronic morphine tolerance by i.c.v. OFQ-Ab. Morphine (5, 10, 20, 40, 50, and 60 mg/kg, s.c., t.i.d.) was given for 6 days. The development of tolerance was assessed following injection of morphine (5 mg/kg, s.c.) at 7:00 a.m. every day using TFL (measured 30 min after the morphine injection). On day 7, all rats were given a test dose (5 mg/kg, s.c.) of morphine. OFQ-Ab ($n = 8$) or normal rabbit serum IgG (normal serum, $n = 8$) was given 20 min prior to morphine injection. TFL was measured for 60 min. Vertical lines represent SEM. **, $P < 0.01$, compared with the normal serum-treated group by ANOVA followed by Newman–Keuls post-hoc test (See ref. 16, Fig. 4).

these animals, i.c.v. injection of OFQ-Ab (1:1 dilution) reversed chronic morphine tolerance by 50% ($P < 0.01$) (Fig. 3). Chronic tolerance to the analgesic effect of EA was produced by repeatedly administering EA of increasing current (1, 2, and 3 mA, each lasting for 10 min, for a total of 30 min) at a frequency of 100 Hz once a day for 6 days. Intracerebroventricular injection of OFQ-Ab (1:1 dilution) in these animals also reversed chronic EA tolerance by 50% ($P < 0.01$) (Fig. 4) [16].

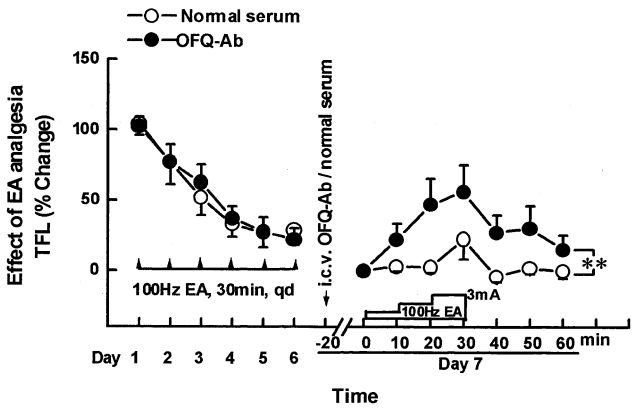

Fig. 4. Reversal of chronic EA tolerance by i.c.v. injection of OFQ-Ab. EA 100 Hz (1, 2, and 3 mA, each for 10 min) was administered once daily for 6 days. The percent increase in TFL was measured at 10-min intervals and averaged as an index of EA analgesia. On day 7, all rats were given 30 min 100 Hz EA. OFQ-Ab ($n = 11$) or normal rabbit serum IgG (normal serum, $n = 10$) was given 20 min before EA stimulation. TFL was then measured for 60 min. Vertical lines represent SEM. **, $P < 0.01$, compared with normal serum-treated group by ANOVA followed by Newman–Keuls post-hoc test (See ref. 16, Fig. 6).

Data presented above were further confirmed by radioimmunoassay of OFQ in the cerebral ventricles of morphine tolerant rats [20]. In this study, we treated rats with increasing doses of morphine for 3 and 5 days, then determined OFQ-immunoreactivity in cerebroventricular perfusates. We clearly demonstrated an increase of 25% and 52% OFQ-immunoreactivity compared to the normal serum control group in the 3 and 5 day morphine-treated groups, respectively [20]. We concluded that continuous administration of high doses of morphine accelerated the biosynthesis and release of OFQ in the brain and that this antagonized the effects of morphine.

5. Concluding statement

Collectively, data obtained by central administration of OFQ or OFQ antibodies suggest that endogenously released OFQ plays an important role in pain modulation, i.e. increased pain sensitivity in the brain and lowered pain sensitivity in the spinal cord.

References

[1] Butour JL, Moisand C, Mollerau C, Meunier JC. [Phe1psi(CH2-NH)Gly2] nociceptin-(1–13)-NH2 is an agonist of the nociceptin (ORL1) receptor. Eur J Pharmacol 1998;349:R5–6.

[2] Carpenter KJ, Dickenson AH. Evidence that [Phe1psi(CH2-NH)Gly2]nociceptin-(1–13)-NH2, a peripheral ORL-1 receptor antagonist, acts as an agonist in the rat spinal cord. Br J Pharmacol 1998;125:949–51.

[3] Chiou LC. [Phe1psi(CH2-NH)Gly2]nociceptin-(1–13)-NH2 activation of an inward rectifier as a partial agonist of ORL1 receptors periaqueductal gray. Br J Pharmacol 1999;128:103–7.

[4] Grisel JE, Mogil JS, Belknap JK, Grandy DK. Orphanin FQ acts as a supraspinal, but not a spinal, anti-opioid peptide. NeuroReport 1996; 7:2125–9.

[5] Grisel JE, Farrier DE, Wilson SG, Mogil JS. [Phe1psi(CH2-NH)Gly2]nociceptin-(1–13)-NH2 acts as an agonist of the orphanin FQ/nociceptin receptor in vivo. Eur J Pharmacol 1998;357: R1–3.

[6] Guerrini R, Calo G, Rizzi A, et al. A new selective antagonist of the nociceptin receptor. Br J Pharmacol 1998;123:163–5.

[7] Han JS, Fei H, Zhou ZF. Met-enkephalin-Arg6-Phe7-like immunoreactive substances mediate electroacupuncture analgesia in the periaqueductal gray of the reabbit. Brain Res 1984;322:289–96.

[8] King MA, Rossia H, Williams L, Pasternak GW. Spinal analgesic activity of orphanin FQ/nociceptin and its fragments. Neurosci Lett 1997;223:113–6.

[9] Mogil JS, Grisel JE, Reinscheid RK, Civelli O, Belknap JK, Grandy DK. Orphanin FQ is a functional anti-opioid peptide. Neuroscience 1996;75:333–7.

[10] Mogil JS, Grisel JE, Zhang G, Belknap JK, Grandy DK. Functional antagonism of μ-, δ- and κ-opioid antinociception by Orphanin FQ. Neurosci Lett 1996;214:1–4.

[11] Quigley DI, Mcdougall J, Darland T, et al. Orphanin FQ is the major OFQ 1–17-containing peptide produced in the rodent and monkey hypothalamus. Peptide 1998;19:133–9.

[12] Rossi GC, Leventhal L, Pasternak GW. Naloxone sensitive orphanin FQ-induced analgesia in mice. Eur J Pharmacol 1996;311:R7–8.
[13] Stanfa LC, Chapman V, Kerr N, Dickenson, AH. Inhibitory action of nociceptin on spinal dorsal horn neurones of the rat, in vivo. Br J Pharmacol 1996;118:1875–7.
[14] Tian JH, Xu W, Fang Y, et al. Bidirectional modulatory effect of orphanin FQ on morphine-induced analgesia: antagonism in brain and potentiation in spinal cord of the rat. Br J Pharmacol 1997;120:676–80.
[15] Tian JH, Xu W, Zhang W, et al. Involvement of endogenous orphanin FQ in electroacupuncture-induced analgesia. NeuroReport 1997;8: 497–500.
[16] Tian JH, Zhang W, Fang Y, Xu W, Grandy DK, Han JS. Endogenous orphanin FQ: evidence for a role in the modulation of electroacupuncture analgesia and the development of tolerance to analgesia produced by morphine and electroacupuncture. Br J Pharmacol 1998; 124:21–6.
[17] Wang YQ, Zhu CB, Cao XD, Wu GC. Supraspinal hyperalgesia and spinal analgesia by [Phe1psi(CH2-NH)Gly2]nociceptin-(1–13)-NH2 in rat. Eur J Pharmacol 1999;376:R1–3.
[18] Xu IS, Wiesenfeld–Hallin Z, Xu XJ. [Phe1psi(CH2-NH) Gly2]nociceptin-(1–13)-NH2, a proposed antagonist of the nociceptin receptor, is a potent and stable agonist in the rat spinal cord. Neurosci Lett 1998;249:127–30.
[19] Xu XJ, Hao JX, Wiesenfeld–Hallin Z. Nociceptin or antinociceptin: potent spinal antinociceptive effect of orphanin FQ/nociceptin in the rat. NeuroReport 1996;7:2092–4.
[20] Yuan L, Han Z, Chang JK, Han JS. Accelerated release and production of orphanin FQ in brain of chronic morphine tolerant rats. Brain Res 1999;826:330–4.