Functional studies using antibodies against orphanin FQ/nociceptin

Jin-Hua Tian, Ji-Sheng Han*

Neuroscience Research Institute, Beijing Medical University, Beijing, China

Received 20 November 1999; accepted 20 March 2000

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. © 2000 Elsevier Science Inc. All rights reserved.

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 neuropeptides 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 [Phe1 89 (CH2-NH) Gly2]NC(1–13)NH2 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-
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-enkephalin (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.

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
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 \((1, 2, \text{and} 3 \, \text{mA, each lasting for} \, 10 \, \text{min, for a total of} \, 30 \, \text{min})\) 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].

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


