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Interactive report

Accelerated release and production of orphanin FQ in brain of chronic morphine tolerant rats [1]

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Abstract

Orphanin FQ has been shown to possess anti-opioid activity at supraspinal level. Our previous work revealed that chronic morphine tolerance could be reversed by intracerebroventricular (i.c.v.) injection of OFQ IgG to rats. In this study, we used radioimmunoassay (RIA) to assess the changes of Orphanin FQ immunoreactivity (OFQ-ir) in cerebroventricular perfusate, periaqueductal gray (PAG) and amygdala of rats made tolerance to morphine (10–60 mg/kg, s.c., t.i.d., for 5 days). The results indicated that: (1) In rats administrated with morphine for 3 and 5 days, the content of OFQ-ir in cerebroventricular perfusate increased by 25% and 52% over the NS control group. (2) The content of OFQ-ir in PAG of rats receiving 1d, 3d and 5d injections of morphine showed an increase of 17%, 48% and 81% respectively over NS group. (3) The content of OFQ-ir in amygdala of rats given 3d and 5d of morphine showed a 36% and 55% increase compared with corresponding control group. It is suggested that continuous use of high doses of morphine accelerated the release and biosynthesis of OFQ in rat brain to antagonize the effect of opioids, which may play a role in the development of morphine tolerance, and that brain OFQ may serve as a delayed negative feedback control on opioid analgesia.

Keywords: Orphanin FQ; Chronic morphine tolerance; Radioimmunoassay; Amygdala; Cerebroventricular perfusate; Periaqueductal gray

1. Introduction

The successful cloning of δ [1,2], μ [3,4] and κ opioid receptors [5,6] quickly led to the discovery of a novel member of the opioid receptor gene family designated orphan opioid like receptor (ORL1) [7] or LC132 [8], Oprl [9], ROR-C [10], MOR-C [11]. The receptor structurally displays approximately 65% identity with μ, κ and δ opioid receptor and is negatively coupled with adenylyl cyclase, but no opioid ligands have been found to interact with this receptor [7,9,10,12,13]. Orphanin FQ (OFQ), the putative endogenous ligand of ORL1, was a newly discovered heptadecapeptide first isolated from porcine hypothalamus in 1995 [14]. While OFQ structurally resembles the existing endogenous opioid peptides, especially dynorphin A (1–17), it does not bind to the classical opioid receptors with high affinity [14]. Much to our surprise, OFQ exhibited a role different from traditional opioids in vivo pharmacological study. The initial behavioral study reported that i.c.v. administration this new neuropeptide in mice produced hyperalgesia in the hot plate test, hence it was named nociceptin according to its apparent pronociceptive properties [15]. Recent studies, however, demonstrated that i.c.v. injection of OFQ could dose-dependently reversed systemic morphine-induced antinociception [16,17], electroacupuncture-induced antinociception [18], opioid-mediated stress-induced antinociception [16] and functionally antagonized the analgesic effect induced by selective opioid receptor agonists DAMGO (μ-selective), DPDPE (δ-selective) and U-50, 488H (κ-selective) in mice [19]. In other words, OFQ acts as an anti-opioid peptide at least at supraspinal level [20].

In situ hybridization and Northern hybridization analysis [21,22] revealed that preproOFQ mRNA is widely distributed and highly expressed in central gray and central tegmental area of the midbrain, amygdala and hypothalamic nuclei. Tian et al. in our laboratory found that OFQ

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IgG administrated cerebroventricularly (i.c.v.) to rats reversed chronic morphine tolerance [23], suggesting that OFQ may play an important role in the development of morphine tolerance. To test the hypothesis that an increased production and release of endogenous OFQ is the possible mechanism of morphine tolerance, we used radioimmunoassay (RIA), a specific and sensitive method for measuring minute amount of neuropeptide, to assess whether there is a change in the amount of OFQ-ir in cerebroventricular perfusate and in the brain tissue of periaqueductal gray (PAG) and amygdala, which contain abundant OFQ [21] and ORL1 transcripts [24].

2. Materials and methods

2.1. Chemicals and animals

Morphine HCl was purchased from Qinhai Drug Company (China). OFQ and OFQ RIA kits were products of Phoenix Pharmaceuticals (USA). Morphine HCl and OFQ were dissolved in normal saline and buffer, respectively. Adult female Wistar rats weighing 200–250 g were provided by the Animal Center in Beijing Medical University. They were housed six in a cage with food pellets and water available ad libitum.

2.2. Nociceptive testing

Experiments were performed in a temperature-controlled room (20 ± 1°C). Nociceptive sensitivity was assessed using the radiant heat tail-flick assay [25]. Rats were kept in a plastic restrainer with hindlimbs and tail extending. Focused light from a 12.5 W projection bulb was applied to the lower 1/3 of the tail and the tail flick latency (TFL) was measured to the nearest 0.1 s. Values from the first 3 measurements, with an interval of 5 min, were averaged as the basal TFL, which was usually in the range of 4–6 s. TFL obtained in subsequent tests was expressed as percentage change from the basal level, with a cut-off limit of 150% in order to prevent possible tissue damage. In every tail flick test, we measured tail temperature, and if it increased more than 1°C (compared with room temperature), the tail flick latency would be corrected by a coefficient of -0.25 s/°C [25].

2.3. Chronic morphine tolerance

To induce morphine tolerance, rats were injected subcutaneously (s.c.) 3 times a day (08:00 h, 16:00 h, 23:00 h) for 5 days with increasing doses of morphine HCl from 10 mg/kg up to 60 mg/kg (10, 20, 40, 50 and 60 mg/kg respectively). In the control group, rats were injected with NS of the same volume instead of morphine for five days. The development of tolerance was monitored every day at 07:00 h by measuring the percentage change of tail-flick latency (TFL) 30 min after the injection of a testing dose of morphine (10 mg/kg, s.c.).

2.4. Cerebroventricular perfusion and extraction of PAG and amygdala

Rats were anesthetized with 10% chlorohydrate (350 mg/kg, i.p.) 20 min after the last injection of morphine or NS in d1, d3 and d5, respectively. Stainless steel cannula (0.8 mm outer diameter) was fixed on the skull unilaterally at stereotaxic coordinates P: 1.1 mm, L: ± 1.5 mm, H: 3.0 mm according to rat brain stereotaxic atlas of Paxinos and Watson [26]. The outlet of the cannula was 3 mm above the skull so as to connect with the tubing from a constant speed pump. Another stainless cannula of 1 cm length (0.8 mm outer diameter) was sharpened and introduced through the incised atlanto-occipital membrane for a depth of 1 mm, and the other end of the cannula was connected to a PE-50 polyethylene tubing of 10 cm length. Artificial cerebrospinal fluid was perfused into the lateral cerebral ventricle at a rate of 1.0 ml/30 min. The perfusate was flowing out through the stainless steel cannula and the PE-50 polyethylene tubing, and was collected in an ice-water bathed Eppendorf tube. After 30 min of perfusion, the rats were rapidly decapitated and the brains were removed promptly and dissected on an ice-cold plate. The PAG and bilateral amygdala were removed according to rat brain stereotaxic atlas [26] with a scalpel, then quickly put into the Eppendorf tube containing 1 ml of 0.5 M acetic acid solution, and heated in a boiling water bath for 10 min. After cooling, the perfusate was centrifuged at 12 000 × g (4°C) for 20 min. The supernatant was frozen and lyophilized, and then kept at -70°C. It was redissolved with 130 ul double distilled water before RIA. The brain tissue was supersonically homogenated at 4°C for 1 min. After 4 h of still setting, the homogenate was centrifuged at 12 000 × g (4°C) for 25 min. The supernatant was stored at -70°C, and reconstituted in equal volume of 0.5 M NaOH solution before the assay. The protein content was assayed by Coomassie Brilliant blue method [27].

2.5. Radioimmunoassay of OFQ-immunoreactivity (OFQ-ir)

The content of OFQ-ir was assayed by RIA using ^{125}I-Tyr-OFQ as radio-ligand. The radioimmunoassay procedure for OFQ in the present study was similar to that suggested by Phoenix Pharmaceuticals. On the basis of 10 consecutive assays, the sensitivity of the assay was 12 pg/ml, and non-special binding was 4.78%, with 8.4% interassay-variations.

2.6. Statistical analysis

The experimental data were expressed as mean ± S.E.M. Group differences were tested by two-way analyses of

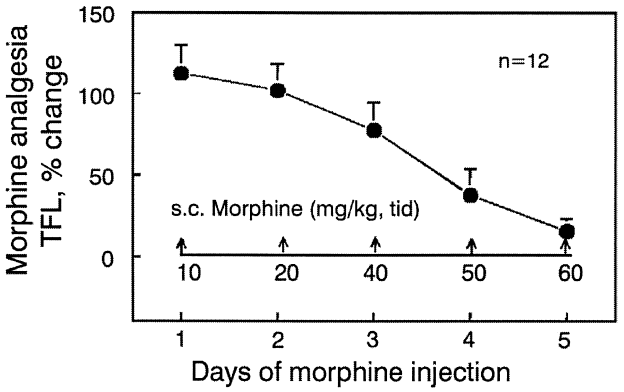

Fig. 1. The development of chronic morphine tolerance. Morphine (10, 20, 40, 50, 60 mg/kg, s.c., t.i.d.) was given for five consecutive days. n = the number of animals. The development of tolerance was tested by a challenging dose of morphine (10 mg/kg, s.c.) administrated everyday at 07:00 h and the TFL was measured 30 min after the morphine injection.

variance (AVOVA) followed by Newman–Keuls post-hoc test. $P < 0.05$ was taken as the significant level of difference.

3. Results

3.1. The development of chronic morphine tolerance in rats

In rats that were given an increasing dose of morphine (10–60 mg/kg, s.c. t.i.d.), the analgesic effect induced by a testing dose of morphine (10 mg/kg) was decreased almost linearly from 113 ± 18% on the 1st day to 78 ± 17% on the 3rd day and 16 ± 7% on the 5th day (Fig. 1). The basal TFL was not significantly changed during this period (4.5 ± 0.1 s and 4.8 ± 0.2 s at the 1st and 5th days of injection, respectively).

3.2. Changes of OFQ-ir in the brain perfusate (BP), PAG and amygdala of chronic morphine tolerance rats

Rats were randomly divided into six groups (n = 11-13/group). Three groups respectively received the 1, 3 or 5d morphine (s.c., t.i.d.), and the other three groups respectively received 1, 3 or 5d NS (s.c., t.i.d.). Each rat was perfusated for 30 min at a rate of 1 ml/30 min after the last injection of morphine or NS. Analyses of variance (ANOVA) showed a significant difference ($F(1, 64) = 14.93$, $p = 0.000263$) between NS and morphine groups. Results followed by Newman–Keuls post-hoc test revealed that, in the control group receiving 1d, 3d or 5d NS, the content of OFQ-ir remained rather stable within the range of 58.7, 55.5 and 56.6 pg/ml (Fig. 2, right). The corresponding value of OFQ-ir in the morphine groups were 63.3, 69.5 and 86.1 pg/ml, respectively, showing an increase of 52% ($p = 0.001$) after 5d morphine injections. In addition, the content of OFQ-ir in rats given 5d morphine was significantly different compared with the value of 1d or 3d morphine injection ($p = 0.025$, $p = 0.007$). The data showed that there was an increase of OFQ-ir content in BP during chronic morphine treatment, presumably representing an increase of OFQ release from the CNS.

At the end of brain perfusation, the PAG and amygdala were quickly dissected, so brain perfusate and brain tissue from PAG and amygdala were obtained from the same a rat to illustrate possible interrelation of biosynthesis and release. ANOVA showed significant difference between

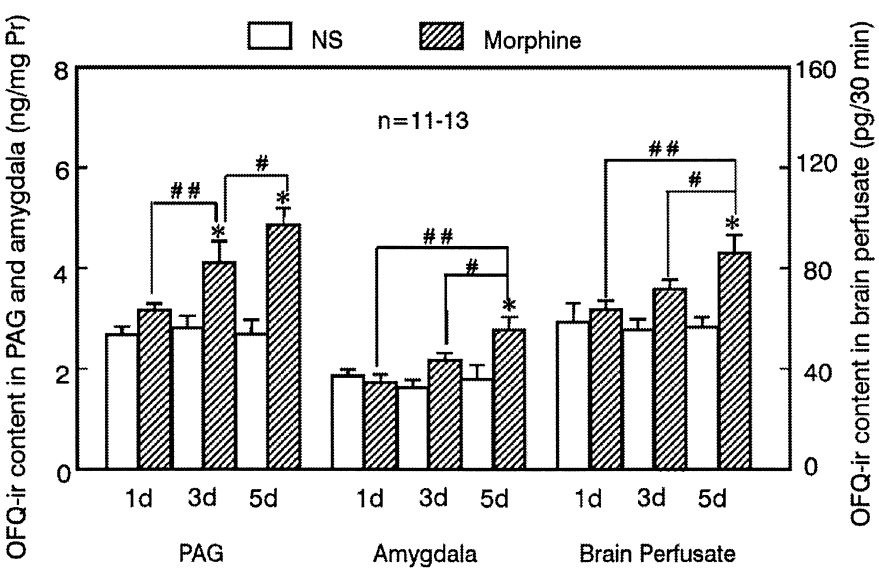

Fig. 2. The changes of OFQ-ir in the brain perfusate (pg/ml), PAG and amygdala (ng/mg protein) of chronic morphine tolerant rats. Data are shown as mean ± S.E.M. of the levels of OFQ-ir. n = the numbers of rats in each group. Left and right Y axis represents OFQ-ir in brain tissue of PAG and amygdala and in brain perfusate, respectively. * $P < 0.01$, compared with corresponding values of NS; # $P < 0.05$, ## $P < 0.01$ compared between two groups indicated by arrows. All the data are assessed by ANOVA followed by Newman–Kuels post-hoc test.

NS and morphine groups ($F(1, 70) = 37.58$, $p = 4.61E\text{-}08$) or group and day ($F(2, 70) = 5.42$, $p = 0.0065$). Results followed by Newman–Keuls post-hoc test revealed that the content of OFQ-ir in the PAG of rats administrated with morphine for 1, 3 and 5 days was 3.12, 4.12 and 4.86 ng/mg Protein (Pr), respectively, as compared with 2.67, 2.80 and 2.70 ng/mg Pr in corresponding NS groups, showing an increase of 17% ($P > 0.05$), 48% ($p = 0.002$) and 81% ($p = 0.0001$). Thus, OFQ-ir content of morphine groups increased gradually with the increase of morphine dose. There was a significant difference between 1d morphine and 3d morphine group ($p = 0.008$), and 3d morphine and 5d morphine group ($p = 0.047$) (Fig. 2, left).

In amygdala, ANOVA also showed significant difference between NS and morphine groups ($F(1, 66) = 8.70$, $p = 0.0044$) or group and day ($F(2, 66) = 4.37$, $p = 0.017$). Results followed by Newman–Keuls post-hoc test revealed that OFQ-ir in the amygdala of rats given 1, 3 and 5 days of morphine was 1.72, 2.21 and 2.79 ng/mg Pr, respectively, as compared with 1.86, 1.63 and 1.80 ng/mg Pr in corresponding control groups, showing a decrease of 7% ($P > 0.05$) and an increase of 36% ($P > 0.05$) and 55% ($p = 0.004$), respectively (Fig. 2, middle). Similarly, OFQ-ir content of amygdala in rats injected with 5d morphine showed significant difference compared with the value of 1d or 3d morphine injection ($p = 0.008$, $p = 0.047$).

4. Discussion

There is a battery of evidence pointing to the conclusion that brain OFQ may play an important role in the mechanism underlying morphine tolerance. This conclusion has been strengthened by the fact that in homozygous mutant mice whose OFQ receptor gene was knocked out, tolerance was not readily induced by systemic morphine injection with a dose of 10 mg/kg, once a day for 5 days [28] . In addition, chronic morphine tolerance induced by multiple injections of morphine (3 times a day for 6 days, with single dose increasing from 5 mg/kg in the 1st day up to 60 mg/kg in the 6th day) could be significantly ($P < 0.01$) although partially (about 50%) reversed by i.c.v. injection of IgG against OFQ [23]. These data seem to suggest that (1) brain OFQ play a role in the development of morphine tolerance, although it is certainly not the sole determinant, (2) The release of central OFQ is markedly accelerated only after several days of morphine treatment. However, no data are available in the literature concerning the time course of the response of brain OFQ to morphine treatment.

In order to observe the biosynthesis and release of brain OFQ in chronic morphine tolerant rats, we measured with RIA the change of OFQ-ir in brain perfusate and in the discrete brain area of PAG and amygdala, which are known to be involved in morphine analgesia [29–31] and are abundantly widely distributed with OFQ and its receptors [21,24]. In fact, it has been shown that microinjection of OFQ into PAG of mice could readily reverse opioid antinociception presumably by inhibiting the efferent neurons [32]. The results of our study clearly indicate that (1) there was a lineal increase of OFQ-ir in the brain perfusate of morphine treated rats, which becomes statistically significant on the 5th day ($P < 0.01$) compared with a rather stable level in the corresponding control group, suggesting a gradual increase of the release of OFQ from brain tissue in the 5 days observation period. (2) There was a concomitant increase in the steady state OFQ-ir level in PAG and amygdala. The reaction in PAG seems to be more rapid and fierce than in amygdala since a significant increase of OFQ-ir in PAG was already seen on the 3rd day of morphine treatment when the dose of morphine was 40 mg/kg (t.i.d.), but not in amygdala where a significant increase of OFQ-ir showed only after 5 days of morphine treatment at a dose of 60 mg/kg (t.i.d.). (3) The increase of OFQ-ir content among morphine groups in brain perfusate, PAG or amygdala rose gradually when the dose of the daily morphine injection increased. Measurement of the content of a neuropeptide in brain tissue reflects an equilibrium of production and release of the neuropeptide. Theoretically, an increase in the brain content of a peptide can be due to an increased biosynthesis or a decreased release. In the present case, however, an accelerated biosynthesis seems more likely since the rate of release has been suggested to be increased.

Taking the endogenously released anti-opioid peptides as negative feedback control for opioids (be endogenously released opioid peptide or exogenously administrated opiates), the speed of the feedback response is of cardinal importance. Cholecystokinin octapeptide (CCK-8), one of the potent anti-opioid neuropeptides, and OFQ seem to represent two extremes of the spectrum. CCK-8 belongs to the category of quick onset and fast fading whose accelerated release immerges as early as the 1st day of morphine injection, but restores to normal level at 5th day [33], whereas OFQ belongs to the category of slow onset and longer lasting whose accelerated production and release starts at 3 days morphine treatment and does not reach plateau after 5 days. While the mechanism of the difference in the dynamic response between the two anti-opioid systems remain to be clarified, understanding of the time course of the reaction may be useful if one intends to design a recipe for overcoming morphine tolerance by the use of antagonists against endogenous anti-opioid peptides.

5. Conclusion

Our results suggests that administration morphine of high dose and long time course may trigger the OFQ system in the central nervous system to exert a delayed negative feedback control, which may constitute one of the

mechanisms of morphine tolerance, especially in the later stage of the development of tolerance.

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