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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

Jin-Hua Tian, Wei Zhang, [1]Yuan Fang, Wei Xu, [1]David K. Grandy & [2]Ji-Sheng Han

Neuroscience Research Institute, Beijing Medical University, Beijing 100083, People's Republic of China; [1]Department of Physiology and Pharmacology, Oregon Health Sciences University, Portland, Oregon 97201, U.S.A.

1 Our previous work has demonstrated that exogenously administered orphanin FQ (OFQ) antagonizes morphine analgesia and electroacupuncture analgesia (EAA) in the brain and potentiates morphine analgesia and EAA in the spinal cord of the rat. In the present study we evaluated the role of endogenously released OFQ in the development of tolerance to morphine and electroacupuncture (EA) and the analgesia produced by electroacupuncture, by use of the IgG fraction of an anti-OFQ antibody (OFQ-Ab) microinjected into the rat central nervous system (CNS).

2 EAA was produced by stimulating rats at a frequency of 100 Hz. Rats were classified as either high responders (HR) or low responders (LR) based on the analgesic effects of EA. LRs could be converted into HRs by the intracerebroventricular (i.c.v.) microinjection of OFQ-Ab at both 1 : 1 and 1 : 10 dilutions but not 1 : 100. HRs could be changed into LRs by the intrathecal (i.t.) injection of OFQ-Ab at both 1 : 1 and 1 : 10 dilutions, but not 1 : 100.

3 Acute morphine tolerance was induced in rats by repeated subcutaneous (s.c.) injections of morphine (5 mg kg, every 2 h) for 16 h. When injected i.c.v. the OFQ-Ab (1 : 1 dilution) had no effect on the development of acute morphine tolerance.

4 Chronic morphine tolerance was produced in rats by repeated injection of morphine (5–60 mg kg, s.c., 3× a day) for 6 days. I.c.v. injection of OFQ-Ab (1 : 1 dilution) reversed this type of morphine tolerance in rats by 50% ($P<0.01$).

5 Acute tolerance to the analgesia produced by EA developed after 6 h of continuous (100 Hz, 3mA) stimulation. This tolerance was almost completely reversed by the i.c.v. injection of OFQ-Ab (1 : 1 dilution) ($P<0.05$).

6 Chronic tolerance to the analgesic effect of EA was produced by repeatedly administering 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. I.c.v. injection of OFQ-Ab (1 : 1 dilution) reversed this kind of tolerance by 50% ($P<0.01$).

7 Together these results suggest that 100 Hz EA may enhance the release of endogenous OFQ in the CNS of the rat, which in turn may act to antagonize EA-produced analgesia in the brain but potentiate EA produced analgesia in the spinal cord. Therefore, OFQ appears to play an important role in the development of tolerance to the analgesic effects produced by EA.

8 The mechanisms underlying the development of acute morphine tolerance and chronic morphine tolerance appear to be different. Central OFQ may play an important role in the development of tolerance after chronic morphine administration.

Keywords: Analgesia; antibody; electroacupuncture; morphine; nociceptin; orphanin FQ; tolerance

Introduction

The novel neuropeptide orphanin FQ (OFQ), also known as nociceptin, was first isolated from rat brain (Meunier *et al*., 1995) and porcine hypothalamus (Reinscheid *et al*., 1995) and has been shown to modulate opioid analgesia (Mogil *et al*., 1996a,b; Grisel *et al*., 1996; Tian *et al*., 1997a) and nociception (Rossi *et al*., 1996 Stanfa *et al*., 1996; Xu *et al*., 1996; King *et al*., 1997). Mogil *et al*. (1996a,b) demonstrated that intracerebroventricular (i.c.v.) injection of OFQ functionally antagonizes opioid-mediated stress-induced analgesia and μ-, δ- and κ- agonist-induced analgesia in mice. Interestingly this effect of OFQ occurs supraspinally but not spinally in mice (Grisel *et al*., 1996). Recently our studies in rats have demonstrated that exogenously administered OFQ antagonizes morphine analgesia in the brain, potentiates morphine analgesia in the spinal cord (Tian *et al*., 1997a), and modulates the analgesia produced by electroacupuncture (EA) (Tian *et al*., 1997b). In another study OFQ administered supraspinally produced an initial hyperalgesic response followed by a delayed analgesia (Rossi *et al*., 1996). Moreover, intrathecal (i.t.) OFQ produced a dose-dependent depression of a spinal nociceptive flexor reflex in the rat (Xu *et al*., 1996). Intrathecally administered nociceptin dose-relatedly inhibited the c-fibre envoked wind-up and post-discharge of dorsal horn neurones of the rat, *in vivo* (Stanfa *et al*., 1996). While King *et al*. (1997) showed that OFQ administered spinally elicited a rapidly appearing, naltrexone-reversible, dose-dependent analgesia without any indication of hyperalgesia. Together these studies suggest a dual role – analgesia versus pro-nociception – for exogenously administered OFQ that is dependent on the site of administration.

[2]Author for correspondence at: Neuroscience Research Institute, Beijing Medical University, Beijing 100083, China.

In order to evaluate the role of endogenously released OFQ in response to the production of analgesia by EA, we have injected different dilutions of a rabbit anti-OFQ antibody (OFQ-Ab) i.c.v. and i.t. This approach has been widely used as a powerful technique to modify selectively the physiological activities of a target peptide. For example, this technique was used to demonstrate that in the central nervous system (CNS) the cholecystokinin octapeptide (CCK-8) plays an important role in the development of morphine tolerance and EA tolerance (Ding *et al*., 1986; Han *et al*., 1986). In the present study we were interested in determining whether repeated injections of morphine, or prolonged EA stimulation, would trigger the release of central OFQ and suppress morphine analgesia or EA produced analgesia. Our results suggest that i.c.v. injection of OFQ-Ab almost completely reversed acute EA tolerance, partially reversed chronic morphine tolerance and EA tolerance, but had no effect on acute morphine tolerance.

Methods

Subjects

Adult female Wistar rats weighing 200–250 g were provided by the Animal Centre, Beijing Medical University. The implantation of i.c.v. cannulae or intrathecal (i.t.) catheters was performed under anaesthesia by using chlorohydrate (300 mg kg^{-1}).

For i.c.v. cannulae, stainless steel tubing (0.8 mm outer diameter) 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*. (1979). Experiments involving i.c.v. injection started 3–4 days after the surgical operation.

Intrathecal (i.t.) catheterization was performed according to the method of Yaksh and Rudy (1976). A PE-10 tube of 13 cm in length was inserted through the incised atlanto-occipital membrane and the dura into the subarachnoid space for 7.5 cm to reach the upper border of the lumbar enlargement. I.t. injections were started one day post surgery. For both types of central injections, the volume administered was 10 μl, delivered over 10 s.

Antibody microinjection

A polyclonal OFQ antiserum was raised in rabbits. Purification of IgG was accomplished by pre-absorption with bovine serum albumin (BSA) and liver acetone powder followed by affinity separation on a protein A column. The purified IgG derived from 1.0 ml antiserum was lyophilized and stored at -70°C, and diluted in 1.0 ml sterile H_2O as 1 : 1 OFQ-Ab. The IgG fraction from non-immunized normal rabbit serum was purified as above and served as control. The OFQ antibody has no cross reactivity with dynorphin A 1–17 or OFQ 1–7 and mouse whole pituitary extract that contains all known forms of endorphin, β-lipotropin and dynorphin (Quigley *et al*., 1997).

OFQ-Ab, as well as normal rabbit serum IgG were administered i.c.v. or i.t. in 1 : 1, 1 : 10 and 1 : 100 dilutions. I.c.v. or i.t. injection of antibody or normal serum was performed 20 min or 30 min ahead of EA stimulation or morphine administration, respectively, allowing the antibody or serum to spread through the brain or spinal cord (Della-Fera *et al*., 1981; Fujimoto *et al*., 1990).

Nociceptive testing

Experiments were performed at room temperature ($20 \pm 1^{\circ}$C). Nociception was evaluated by radiant heat tail-flick test (Ren & Han, 1979). 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 recorded 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 cutoff limit of 150% in order to avoid any 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 $^{\circ}C^{-1}$ (Ren & Han, 1979).

Morphine tolerance

Acute morphine tolerance Rats received eight consecutive injections of morphine (5 mg kg^{-1}, s.c.) at 2 h intervals. Nociception was assessed 30 min after each injection by radiant-heat tail flick assay as an index of morphine analgesia.

Chronic morphine tolerance Morphine was injected s.c. three times a day (8 h 00 min, 15 h 00 min, 22 h 00 min) for six days. The dose of morphine was 5, 10, 20, 40, 50, 60 mg kg^{-1} (3 times day^{-1}) from day 1 to day 6, respectively. The development of tolerance was detected every day at 7 h 00 min by measuring tail flick latency 30 min after a challenge injection of morphine (5 mg kg^{-1}, s.c.).

Electroacupuncture (EA) and tolerance to analgesia produced by EA

EA experiment To produce electroacupuncture analgesia (EAA) electrical stimulation was administered via stainless steel needles inserted 5 mm at two sites on the hind legs: one at the 'Zusanli' point (ST36) near the knee joint (5 mm lateral to the anterior tubercle of tibia) and the other at the 'Sanyinjiao' point (SP6) near ankle joint (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 'HANS' 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 of 0–3 mA and a frequency of 100 Hz. The intensity was set at 1 mA and then increased stepwise to 2 mA and then 3 mA, each of which lasted for 10 min. TFL was determined at 10, 20 and 30 min during EA stimulation, and for additional 30 min following the cessation of EA stimulation (again, at 10 min intervals).

Acute EA tolerance Rats were given continuous EA stimulation (100 Hz, 3 mA) for 6 h and antinociception in these rats was tested every hour , which was used as an index of EA tolerance.

Chronic EA tolerance EA stimulation (1, 2, 3 mA, each for 10 min) as described in 'EA experiment' was given once a day (8 h 00 min) for 6 days. Each time, TFL was measured every 10 min during the 30 min EA stimulation. The mean value was used to document the development of EA tolerance.

Chemicals

OFQ antibody was provided by Dr David K. Grandy (Oregon Health Sciences University, U.S.A.). Morphine HCl is a product of Qinghai Drug House (China), dissolved in sterile normal saline (NS).

Statistical analysis

The data are expressed as the mean ± s.e.mean. Group differences were assessed by two-way analyses of variance (ANOVAs) followed by Newman-Keuls *post-hoc* test. $P<0.05$ was taken as the significant level of difference.

Results

The effect of i.c.v. OFQ-Ab on the analgesia produced by 100 Hz EA

The results of a pilot study suggested that the OFQ antibody tended to increase the efficacy of 100 Hz EAA when injected i.c.v. and to suppress 100 Hz EAA after i.t. injection. We have also recently demonstrated that outbred rats display different degrees of analgesia (Tang *et al*., 1997). Therefore, to determine whether the OFQ-Ab is able to modify the analgesia produced by 100 Hz EA, 80 rats were tested and classified as either low responders (LR, increase of mean TFL less than 60% during the 30 min-EA stimulation) or high responders (HR, increase of mean TFL more than 60 %) at least one week before the antibody microinjection experiments.

Forty-seven LR rats were randomly divided into four groups, individually re-assessed for their baseline nociceptive sensitivity and then given i.c.v. injections (1 : 100, 1 : 10, 1 : 1 OFQ-Ab or 1 : 1 normal rabbit serum IgG), respectively. Twenty minutes later, they were given 100 Hz EA as described in the Methods. Individual TFLs were assessed over a 60 min test period. As shown in Figure 1, 100 Hz EA increased TFL in the control group by only 33.8%. In contrast the OFQ-Ab (1 : 10 and 1 : 1 dilutions) significantly increased TFL by 52.4% and 76.3% ($P<0.05$ and $P<0.01$, respectively).

The effect of i.t. OFQ-Ab on the analgesia produced by 100 Hz EA

In this experiment, we used 43 HR rats which were randomly divided into four groups of 10–11 rats, each receiving 1 : 100, 1 : 10, 1 : 1 dilutions of OFQ-Ab or normal rabbit serum IgG, respectively. The i.t. injection of IgG was made 30 min before 100 Hz EA stimulation. As shown in Figure 2, 100 Hz EA increased TFL by 63.3% in normal serum treated rats. OFQ-Ab (1 : 10 and 1 : 1 dilutions) significantly reduced EAA (mean TFL in 60 min were 24.4% and 18.2%, respectively, $P<0.01$).

I.c.v. OFQ-Ab failed to reverse acute morphine tolerance

For this study, seventeen rats were randomly divided into two groups (normal serum IgG group and OFQ-Ab group). All rats were given morphine (5 mg kg^{-1}, s.c.) every 2 h for 16 h to induce an acute tolerance to morphine analgesia. Challenge doses of morphine (5 mg kg^{-1}, s.c.) were given 2, 6 and 10 h after the 8th injection. OFQ-Ab or normal serum IgG were injected i.c.v. 20 min before the 1st challenge dose of morphine. As shown in Figure 3, no significant difference was found between the two groups, indicating that the OFQ

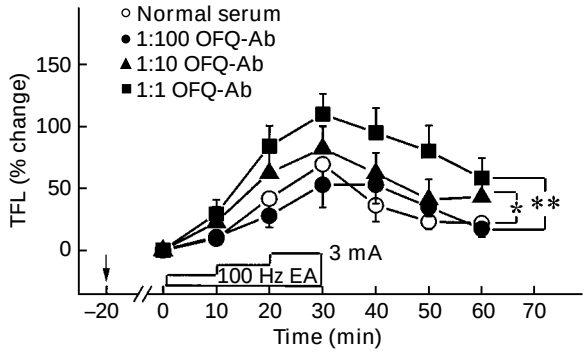

Figure 1 OFQ-Ab dose-dependently augmented 100 Hz EA stimulation-induced analgesia in LR rats. OFQ-Ab or normal rabbit serum IgG (normal serum) were given i.c.v. 20 min before EA stimulation. Normal serum ($n=12$), 1 : 100 OFQ-Ab ($n=12$), 1 : 10 OFQ-Ab ($n=12$) and 1 : 1 OFQ-Ab ($n=11$) were injected, i.c.v., at the arrow. Vertical lines represent s.e.mean. $*P<0.05$, $**P<0.01$, compared with normal serum-treated group, tested by ANOVA followed by Newman-Keuls *post-hoc* test.

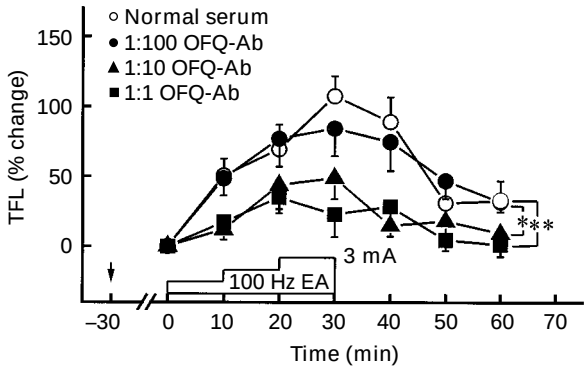

Figure 2 OFQ-Ab dose-dependently inhibited 100 Hz EA stimulation-induced analgesia in HR rats. OFQ-Ab or normal rabbit serum IgG (normal serum) were given i.t. 30 min before EA stimulation. Normal serum ($n=11$), 1 : 100 OFQ-Ab ($n=11$), 1 : 10 OFQ-Ab ($n=10$) and 1 : 1 OFQ-Ab ($n=11$) were injected, i.t., at the arrow. Vertical lines represent s.e.mean. $**P<0.01$, compared with normal serum-treated group, tested by ANOVA followed by Newman-Keuls *post-hoc* test.

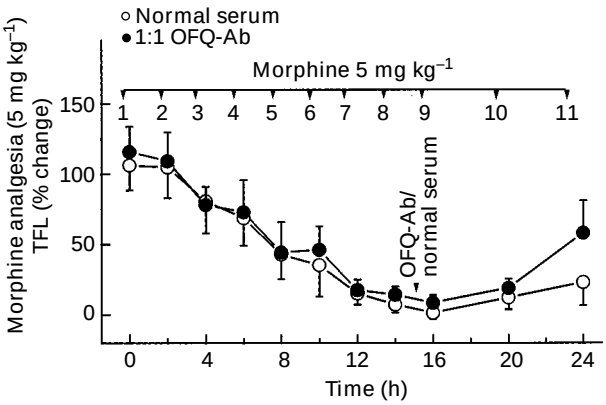

Figure 3 Effect of OFQ-Ab microinjection (i.c.v.) on the development of acute morphine tolerance. All rats were given morphine (5 mg kg^{-1}, s.c.) every 2 h for 16 h, followed by a challenge dose of morphine (5 mg kg^{-1}, s.c.) 2, 6, 10 h after the 8th injection. OFQ-Ab ($n=8$) or normal rabbit serum IgG (normal serum, $n=9$) was given 20 min before the 1st challenge dose of morphine. Tail flick latency (TFL) was assessed 30 min after each injection of morphine. Vertical lines represent s.e.mean. $P>0.05$, compared with normal serum-treated group, tested by ANOVA followed by Newman-Keuls *post-hoc* test.

antibody was not able to reverse an acute tolerance to morphine analgesia.

Reversal of tolerance to the analgesic effects of chronic morphine administration by i.c.v. OFQ-Ab

A group of 16 rats was given repeated injections of morphine (5–60 mg kg^{-1}, s.c., t.i.d.) for 6 days to induce chronic-morphine tolerance. On day 7 the rats were evenly divided into two groups to receive either OFQ-Ab or normal serum IgG, respectively. Twenty minutes after the i.c.v. injection of antibody a test dose of morphine (5 mg kg^{-1}) was given to all rats. TFLs were measured for 60 min. As shown in Figure 4, morphine analgesia was increased by 50% in the OFQ-Ab-treated group ($P<0.01$).

Reversal of tolerance to the analgesic effects of acute EA by i.c.v. OFQ-Ab

Continuous EA (100 Hz, 3 mA) for 6 h produced tolerance in a group of 16 rats as determined by the gradual decrease in the production of analgesia (Figure 5). This group was then subdivided in two: one received OFQ-Ab the other normal serum IgG 40 min after the cessation of EA stimulation. All rats were given a challenge session of 100 Hz EA (3 mA, 10 min) 1, 4, 8 h after the end of 6 h EA. TFLs were measured at the termination of each 10 min EA session. EA tolerance was almost completely reversed by OFQ-Ab ($P<0.05$) within 3 h, whereas in the normal serum IgG group the recovery was slow and incomplete during a period of 7 h (Figure 5).

Reversal of tolerance to analgesic effects of chronic EA by i.c.v. OFQ-Ab

EA stimulation of 100 Hz (1, 2, 3 mA, each lasting for 10 min) was given to a group of 21 rats once a day for 6 days to induce tolerance to the analgesia produced by chronic EA. On day 7, 20 min before 100 Hz EA stimulation rats received an i.c.v. injection of either normal serum IgG (10 rats) or OFQ-Ab (11 rats). TFLs were evaluated over a 60 min test period. A reversal of tolerance to EAA was observed in the rats receiving OFQ-Ab, but not in rats receiving normal serum ($P<0.01$, Figure 6).

Discussion

The recent literature describes apparently paradoxical effects of OFQ on pain modulation, i.e., analgesia in the spinal cord and pronociception in the brain. This situation is not unique to OFQ. Several other agents, such as morphine (Kayser *et al*., 1987; Crain & Shen, 1990), dynorphin (Ren *et al*., 1985; Dickenson & Knox, 1987; Fujimoto *et al*., 1990), and naloxone (Taiwo *et al*., 1989), also produce paradoxical effects under certain circumstances.

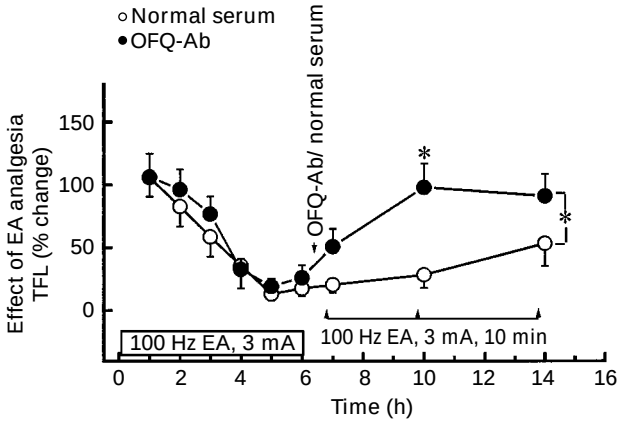

Figure 5 Reversal of acute EA tolerance by i.c.v. injection of OFQ-Ab. Continuous EA (100 Hz, 3 mA) was given for 6 h. TFL was measured at the end of each hour. All rats were given a test session of EA (100 Hz, 3 mA, 10 min) 1, 4, 8 h after the cessation of EA. OFQ-Ab ($n=8$) or normal rabbit serum IgG (normal serum, $n=8$) was given 20 min before the 1st challenge of EA. Vertical lines represent s.e.mean. $^*P<0.05$, compared with normal serum-treated group, assessed by ANOVA followed by Newman-Keuls *post-hoc* test.

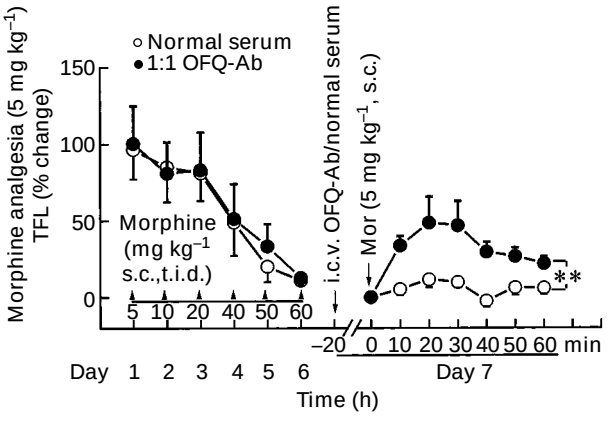

Figure 4 Reversal of chronic morphine tolerance by i.c.v. injection of OFQ-Ab. Morphine (5, 10, 20, 40, 50, 60 mg kg^{-1}, s.c., t.i.d.) was given for 6 days. The development of tolerance was tested by injection of morphine (5 mg kg^{-1}, s.c.) at 7h 00min every day and the TFL was measured 30 min after the morphine injection. On day 7, all rats were given a test dose (5 mg kg^{-1}, s.c.) of morphine. OFQ-Ab ($n=8$) or normal rabbit serum IgG (normal serum, $n=8$) was given 20 min before morphine injection. TFL was measured for 60 min. Vertical lines represent s.e.mean. $^{**}P<0.01$, compared with normal serum treated group, tested by ANOVA followed by Newman-Keuls *post-hoc* test.

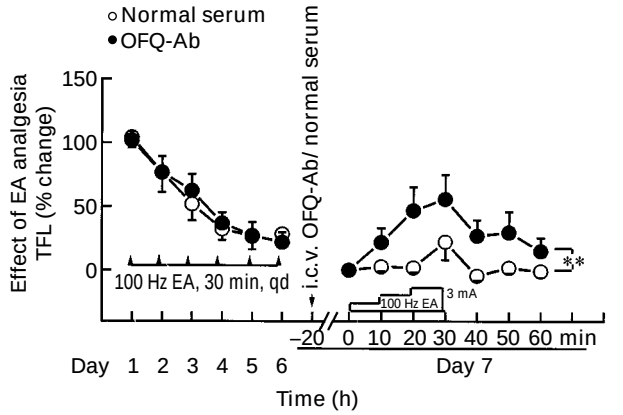

Figure 6 Reversal of chronic EA tolerance by i.c.v. injection of OFQ-Ab. EA 100 Hz (1, 2, 3 mA, each for 10 min) was given once a day for 6 days. The % increase of TFL was measured at 10 min intervals and averaged as the index of EA analgesia. On day 7, all rats were given one session of 30 min 100 Hz EA. OFQ-Ab ($n=11$) or normal rabbit serum IgG (normal serum, $n=10$) was given 20 min before EA stimulation. TFL was measured for 60 min. Vertical lines represent s.e.mean. $^{**}P<0.01$, compared with normal serum-treated group, assessed by ANOVA followed by Newman-Keuls *post-hoc* test.

Rationale for using antibody microinjection technique for the evaluation of endogenous OFQ in CNS

Since the effects of the OFQ which seem 'paradoxical' may be due to the extreme doses being used and/or the route of administration, we decided to study the role of endogenously released OFQ under physiological conditions. Since a selective antagonist for the orphan opioid-like receptor (ORL_1) is still lacking we used the antibody microinjection technique which has been proven to be a reliable means of selectively inhibiting/blocking the functional activity of a given neuropeptide (Della-Fera *et al*., 1981; Schulz *et al*., 1981; Carr *et al*., 1987; Fujimoto *et al*., 1990).

Although it seems that the site of action of an antibody microinjected into the lateral ventricle would be restricted mainly to superficial layers of the ventricular wall, deeper penetration of brain tissue by antibodies has been demonstrated both morphologically (Han, 1987; Ma *et al*., 1992) and functionally (Della-Fera *et al*., 1981; Schulz *et al*., 1981; Carr *et al*., 1987; Fujimoto *et al*., 1990). However, in order to control the non-specific action of the antiserum, 5 precautions were taken: (1) the IgG component rather than the whole antiserum was used; (2) the IgG component of normal rabbit serum was used as a control; (3) a series of dilutions of the IgG, rather than a fixed dose, was used; (4) a fixed volume of 10 μl for i.c.v. and i.t. injection was used; (5) the OFQ antiserum preparation shows virtually no cross-reactivity with (β-endorphin, dynorphin1-17 or OFQ1-7 (Quigley *et al*., 1997).

While leakage of the OFQ antibody either from the lateral ventricle to the spinal fluid or via a regurgitation from the spinal subarachnoid space to the supraspinal level cannot be completely ruled out, if it does occur it does not seem to be of importance since we have demonstrated the effects induced by injection of OFQ in supraspinal and spinal levels are completely opposite in direction (Tian *et al*., 1997a).

Possible role for endogenously released OFQ in mediating EA analgesia

Our previous findings of the bidirectional modulatory effects of OFQ on morphine analgesia (Tian *et al*., 1997a) and EA analgesia (Tian *et al*., 1997b) have since been confirmed by others (Mogil *et al*., 1996a,b; Grisel *et al*., 1996; Stanfa *et al*., 1996; Xu *et al*., 1996; King *et al*., 1997). In the present study this duality was further tested by injecting OFQ antibody into the cerebral ventricle and spinal subarachnoid space, to assess whether EA analgesia could be increased by OFQ-Ab i.c.v. injection and decreased by i.t. injection.

Previous studies have shown that rats can be divided into two 'groups' according to their response to standard EA stimulation (100 Hz, increasing intensity of 1-2-3 mA for a total of 30 min) (Tang *et al*., 1997): rats demonstrating an increase of TFL over 60% are categorized as 'high responders' (HR), while those showing an increase of TFL less than 60% are categorized as 'low responders' (LR). If OFQ does exert a functional anti-opioid effect in brain and an analgesic effect in the spinal cord one might expect an OFQ antibody given i.c.v. to be able to change a LR into HR. Similarly, the i.t. injection of an OFQ antibody might change a HR rat into a LR. Both of these predictions were substantiated by our data (Figures 1 and 2). The present results suggest that 100 Hz EA may enhance the release of endogenous OFQ in the brain of LR rats since blockade of the actions of OFQ by the i.c.v. injection of an OFQ antibody changed LR into HR. The finding that HR rats could be changed into LR rats following an i.t. injection of an OFQ antibody suggests that endogenous OFQ also plays an important role in mediating 100 Hz EA analgesia in the spinal cord of HR rats.

Involvement of OFQ in the development of tolerance to morphine and EA

If brain OFQ functions as an anti-opioid peptide, one might also predict that OFQ may play a role in the development of the tolerance that results after repeated administration of morphine or EA. The results, depicted in Figure 4, suggest that chronic morphine tolerance induced by a 6 day regimen of morphine injections can be reversed significantly ($P<0.01$) albeit partially (about 50%), by the i.c.v. injection of an OFQ antibody. A similar reversal (about 50%, $P<0.01$) of the tolerance that developed in response to chronic EA (one session per day, lasting for 30 min for 6 consecutive days) was produced by the i.c.v. injection of OFQ IgG (Figure 6). These results are consistent with the hypothesis that the continuous exposure of the CNS to a higher than normal concentration of opioids (either exogenously injected or endogenously released) for 6 days may trigger an increase release of OFQ, which in turn may act to reduce the efficacy of opioid-mediated analgesia.

In another experimental design when EA was administered continuously for 6 h a significant degree of acute tolerance developed (Figure 5). As demonstrated in previous studies, the spontaneous recovery from such acute tolerance takes about 24 h (Han *et al*., 1981). This can also be seen in Figure 5, where a slow recovery took place in the control group that received IgG obtained from normal rabbit serum. In contrast, when OFQ antibody IgG was administered i.c.v., this process of recovery was markedly accelerated suggesting that brain OFQ may have been recruited to function as an anti-opioid within a period of 6 h.

Acute tolerance to morphine analgesia was clearly produced by a regimen in which 8 injections of morphine were given over a 16 h period (Figure 3). It is interesting to note that this type of acute morphine tolerance could not be reversed by the i.c.v. injection of OFQ antibody. One interpretation of this result is that brain OFQ is not able to affect acute morphine tolerance. The possibility that the acute tolerance that develops to morphine given repeatedly over a relatively short time period does not involve the release of OFQ in the brain suggests acute and chronic morphine tolerance are qualitatively different. Our further understanding of this process will greatly benefit from the use of an antagonist of the OFQ receptor.

Since acupuncture has been shown to activate central endophin systems, the similarities between acupuncture and morphine analgesia are plain and readily explicable. However, morphine cannot substitute for acupuncture because the latter activates multiple neural pathways, leading to altered activity in numerous CNS systems (Han & Terenius, 1982). Thus, morphine tolerance may share some similarities with EA tolerance, but they are not identical.

In conclusion, the present results suggest that brain OFQ may be partially responsible for the low responder phenotype in rats, while spinal OFQ may be an important factor in the high responder phenotype. In addition this study provides support for the hypothesis that OFQ in the brain may be an important anti-opioid peptide in the development of tolerance to opioid analgesia and electroacupuncture produced analgesia.

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References

CARR, K.D., BAK, T.H., GIOANNINIA, T.L. & SIMON, E.J. (1987). Antibodies to dynorphin A (1-13) but not β-endophin inhibit electrically elicited feeding in the rat. *Brain Res.,* **422,** 384 – 388.

CRAIN, S.M. & SHEN, K-F. (1990). Opioids can evoke direct receptor-mediated excitatory effects on sensory neurons. *Trends Pharmacol. Sci.,* **11,** 77 – 80.

DELLA-FERA, M.A., BAILE, C.A., SCHNEIDER, B.S. & GRINKER, J.A. (1981). Cholecystokinin antibody injected in cerebral ventricles stimulates feeding in sheep. *Science,* **212,** 687 – 689.

DICKENSON, A.H. & KNOX, R.J. (1987). Antagonism of μ-opioid receptor-mediated inhibitions of nociceptive neurones by U50,488H and dynorphin A1-13 in the rat dorsal horn. *Neurosci. Lett.,* **75,** 229 – 234.

DING, X.Z., FAN, S.G., ZHOU, J.P. & HAN, J.S. (1986). Reversal of tolerance to morphine but no potentiation of morphine-induced analgesia by antiserum against cholecytokinin octapeptide. *Neuropharmacology,* **25,** 1155 – 1160.

FUJIMOTO, J.M., ARTS, K.S., RADY, J.J. & TSENG, L.F. (1990). Spinal dynorphin A (1-17): possible mediator of antianalgesic action. *Neuropharmacology,* **29,** 609 – 617.

GRISEL, J. E., MOGIL, J. S., BELKNAP, J. K. & GRANDY, D. K. (1996). Orphanin FQ acts as a supraspinal, but not a spinal, anti-opioid peptide. *NeuroReport,* **7,** 2125 – 2129.

HAN, J.S. (1987). Antibody microinjection: a new approach for studying the functions of neuropeptides. *Chin. Med. J.,* **100,** 459 – 464.

HAN, J.S., DING, X.Z. & FAN, S.G. (1986). Cholecystokinin octapeptide (CCK-8): antagonism to electroacupuncture analgesia and a possible role in electroacupuncture tolerance. *Pain,* **27,** 101 – 115.

HAN, J.S., LI, S.J. & TANG, J. (1981). Tolerance to electroacupuncture analgesia and its cross tolerance to morphine. *Neuropharmacology,* **20,** 593 – 596.

HAN, J.S. & TERENIUS, L. (1982). Neurochemical basis of acupuncture analgesia. *Ann. Rev. Pharmacol. Toxicol.,* **22,** 193 – 220.

KAYSER, V., BESSON, J.M. & GUILBAUD, G. (1987). Paradoxical hyperalgesic effect of exceedingly low doses of systemic morphine in an animal model of persistent pain (Freund's adjuvant-induced arthritic rats). *Brain Res.,* **414,** 155 – 157.

KING, M.A., ROSSIA, H., WILLIAMS, L. & PASTERNAK, G.W. (1997). Spinal analgesic activity of orphanin FQ/nociceptin and its fragments. *Neurosci. Lett.,* **223,** 113 – 116.

MA, Q.P., SHI, Y.S. & HAN, J.S. (1992). Intrathecally injected antibody can diffuse into spinal cord. *Intern. J. Neurosci.,* **65,** 155 – 159.

MEUNIER, J.-C., MOLLEREAU, C., TOLL, L., SUAUDEAU, C., MOISAND, C., ALVINERIE, P., BUTOUR, J.-L., GUILLEMOT, J.-C., FERRARA, P., MONSARRAT, B., MAZARGUIL, H., VASSART, G., PARMENTIER, M. & COSTENTIN, J. (1995). Isolation and structure of the endogenous agonist of opioid receptor-like ORL1 receptor. *Nature,* **377,** 532 – 535.

MOGIL, J.S., GRISEL, J.E., REINSCHEID, R.K., CIVELLI, O., BELKNAP, J.K. & GRANDY, D.K. (1996a). Orphanin FQ is a functional anti-opioid peptide. *Neuroscience,* **75,** 333 – 337.

MOGIL, J.S., GRISEL, J.E., ZHANG, G., BELKNAP, J.K. & GRANDY, D.K. (1996b). Functional antagonism of μ-, δ- and κ-opioid antinociception by Orphanin FQ. *Neurosci. Lett.,* **214,** 1 – 4.

PELLEGRINO, L.J., PELLEGRINO, A.S. & CUSHMAN, A.J. (1979). *A Stereotaxic Atlas of Rat Brain, 2nd Ed.* New York: Plenum.

QUIGLEY D.I., MCDOUGALL, J., DARLAND, T., ZHANG, G., RONNEKLEIV, O.K., GRANDY, D.K. & ALLEN, R. G. (1997). Orphanin FQ^{1-17} (OFQ^{1-17}) is the major OFQ-containing peptide produced in the rodent and monkey hypothalamus. *Peptide* **19,** 133 – 139.

REINSCHEID, R.K., NOTHACKER, H.-P., BOURSON, A., ARDATI, A., HENNINGSEN, R.A., BUNZOW, J.R., GRANDY, D.K., LANGEN, H., MONSMA, F.J. & CIVELLI, O. (1995). Orphanin FQ: a novel neuropeptide which is a natural ligand of an opioid-like G protein-coupled receptor. *Science,* **270,** 792 – 794.

REN, M.F. & HAN, J.S. (1979). Rat tail flick acupuncture analgesia model. *Chin. Med. J.,* **92,** 567 – 582.

REN, M.F., LU, C.H. & HAN, J.S. (1985). Dynorphin-A-(1-13) antagonizes morphine analgesia in the brain and potentates morphine analgesia in the spinal cord. *Peptides,* **6,** 1015 – 1020.

ROSSI, G.C., LEVENTHAL, L. & PASTERNAK, G.W. (1996). Naloxone sensitive orphanin FQ-induced analgesia in mice. *Eur. J. Pharmacol.,* **311,** R7 – R8.

SCHULZ, P., WILHELM, A., PIRKE, K.M., GRAMSCH, C. & HERZ, A. (1981). β-Endophin and dynorphin control serum luteinizing hormone level in immature female rats. *Nature,* **294,** 757 – 759.

STANFA, L.C., CHAPMAN, V., KERR, N. & DICKENSON, A.H. (1996). Inhibitory action of nociceptin on spinal dorsal horn neurones of the rat, *in vivo*. *Br. J. Pharmacol.,* **118,** 1875 – 1877.

TAIWO, Y.O., BASBAUM, A.I., PERRY, F. & LEVINE, J.D. (1989). Paradoxical analgesia produced by low doses of the opiate-antagonist naloxone is mediated by interaction at a site with characteristics of the delta opioid receptor. *J. Pharmacol. Exp. Ther.,* **249,** 97 – 100.

TANG, N.M., DONG, H.W., WANG, X.M., TSUI, Z.C. & HAN, J.S. (1997). Cholecystokinin antisense RNA increases the analgesic effect induced by electroacupunture or low dose morphine: conversion of low responder rats into high responders. *Pain,* **71,** 71 – 80.

TIAN, J.H., XU, W., FANG, Y., MOGIL, J.S., GRISEL, J.E., GRANDY, D.K. & HAN, J.S. (1997a). Bidirectional modulatory effect of orphanin FQ on morphine-induced analgesia: antagonism in brain and potentiation in spinal cord of the rat. *Br. J. Pharmacol.,* **120,** 676 – 680.

TIAN, J.H., XU, W., ZHANG, W., FANG, Y., GRISEL, J.E., MOGIL, J.S., GRANDY, D.K. & HAN, J.S. (1997b). Involvement of endogenous orphanin FQ in electroacupuncture-induced analgesia. *NeuroReport,* **8,** 497 – 500.

XU, X.J., HAO, J.X. & WIESENFELD-HALLIN, Z. (1996). Nociceptin or antinociceptin: potent spinal antinociceptive effect of orphanin FQ/nociceptin in the rat. *NeuroReport,* **7,** 2092 – 2094.

YAKSH, T.L. & RUDY, T.A. (1976). Chronic catheterization of the spinal subarachnoid space. *Physiol. Behav.,* **17,** 1031 – 1036.

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