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Antagonistic effect of orphanin FQ on opioid analgesia in rat[1]

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KEY WORDS orphanin FQ; nociceptors; opioid analgesics; mu opioid receptor; delta opioid receptor; kappa opioid receptor

AIM: To study the effect of orphanin FQ (OFQ), a newly discovered heptadecapeptide, on nociception and opioid analgesia. **METHODS**: The intracerebroventricular (icv) and intrathecal (ith) injections were used to give the drugs. The tail-flick model of rats were used to test the pain threshold. **RESULTS**: OFQ (icv or ith) 0.1 μg had no effect on nociception but 0.5 – 10 μg induces hyper-reaction of rat to noxious electric stimulus; the decapeptide (OFQ_{1-10} icv), a fragment of the OFQ, did not affect the pain reaction of rats. Fentanyl (1 μg, icv or ith), a selective μ-receptor agonist, DSLET (5 μg, icv or ith), a selective δ-receptor agonist, or U50488H (1 μg, ith), a κ-receptor agonist, induced an increase in pain threshold, when OFQ (0.1 or 1 μg) was added together with one of them (except for the ith injection of DSLET), the increase of pain threshold was reduced obviously. **CONCLUSION**: OFQ induces hyperalgesia and antagonizes opioid analgesia mediated by μ- and δ-receptors in the brain and by μ- and κ- but not δ-receptors in the spinal cord of rats.

Since the cloning of μ, δ, and κ opioid receptors were reported, several laboratories have reported a novel orphan opioid receptor clone (LC132 or ORL1) which is distinct from the established opioid receptors in pharmacological profiles[1-4]. The novel orphan receptor does not bind any of the known opioid ligands with satisfactory affinity. An endogenous 17-amino acid peptide was discovered by two independent laboratories[5-6]. The chemical structure is FGGFTGARKSARKLANQ. It bounds to LC132 or ORL1 receptor with high affinity and inhibits forskolin-stimulated adenylyl cyclase activity. This peptide was proposed to be the natural ligand of the orphan receptor, and was named as "Orphanin FQ (OFQ)"[6].

The OFQ is similar to other endogenous opioid peptide (EOP), particularly dynorphin in structure, but distinct from EOP in pain modulation. It elicits hyperalgesia in mice[5-6]. The present study was designed to investigate the effect of OFQ on rat's response to noxious electric stimulus and opioid analgesia in tail-flick model, so as to get a better knowledge of the function of OFQ in the central nervous system.

MATERIALS AND METHODS

Materials Sprague-Dawley ♂ rats (180 – 240 g, Certificate No 02-22-2) were supplied by the Experimental Animal Center, Shanghai Medical University. The heptadecapeptide OFQ and OFQ_{1-10} (a decapeptide, one of the fragment of OFQ) were synthesized and purified in Shanghai Institute of Biochemistry, Chinese Academy of Sciences (Applied Biosystems 430A peptide synthesiser and BOC-Gln-PAM-resin were used. The crude peptide was sequentially purified on columns of Sephadex G-10 and HPLC. Its amino acid composition was consistent with the theoretical values). *N*-phenyl-*N*-[1-(2-phenylethyl)-4-piperidinyl] propanamide (fentanyl, Fen, M_r 528.60), a μ-opioid receptor agonist, and trans-(±)-3,4-dichloro-*N*-methyl-*N*-(2-[1-pyrrolidinyl] cyclohexyl)-benzeneacetamide (U50488H, M_r 465.40), a κ-opioid receptor agonist, were obtained from Sigma Chemical Co (USA), [*D*-Ser2]-Leu-Enkephalin-Thr (DSLET, M_r 586.85), a δ-opioid receptor agonist, was purchased from Peninsula Laboratories Inc (USA); double-headed arrowhead protease inhibitor[7], was the product of the Shanghai Institute of Biochemistry.

Intracerebroventricular (icv) injection of drugs The treatment of the rats conformed to the guidelines of International Association for the Study of Pain[8]. Implantation of the cannula was performed stereotaxically under anesthesia

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with sodium pentobarbital (30 mg·kg^{-1}, ip)[9]. Experiment with icv injection was performed 24 – 48 h after operation. The drugs were dissolved in sterilized normal saline (NS). The OFQ solution was added with arrowhead protease inhibitor 1 g·L^{-1} for preventing from proteolysis after injection.

Intrathecal (ith) injection of drugs Rats were anesthetized with sodium pentobarbital (30 mg·kg^{-1}, ip). PE-10 polyethylene catheter of 75 mm long was implanted through atlanto-occipital membrane down to the lumbar enlargement of the spinal cord[10]. Experiment with ith injection of drugs were carried out 24 – 48 h after operation. Drugs were injected via the ith catheter at a volume of 10 μL, followed by 10 μL of NS for flushing.

Measurement of pain threshold Rats were kept in special-made holders for tail-flick test. Room temperature was kept at 20 ± 1 ℃. Pain threshold was measured with *WQ-9E* Pain threshold Meter (Beijing)[11]. The steadily increasing current was applied as noxious stimulus via needle electrodes connected with the Meter. The smallest intensity of the current provoking the tail-flick was recorded as pain threshold. The mean value of basal pain threshold in normal rats ranged from 150 to 200 μA. The pain threshold was measured successively after drug injection at the interval of 10 min and the values of the measurements were expressed as net changes (increment or decrement) from the basal level. Data were assessed by ANOVA followed by *Q*-test.

RESULTS

Effect of icv injection of OFQ on pain threshold and opioid analgesia

Inducing decrement of pain threshold in rat tail-flick test Rats ($n = 31$) were given icv injection of NS (20 μL, $n = 4$, as control), OFQ 0.1 μg ($n = 4$), OFQ 0.5 μg ($n = 4$), OFQ 1 μg ($n = 8$), OFQ 10 μg ($n = 5$), and OFQ_{1-10} (1 μg, $n = 6$). The pain threshold showed no obvious change after icv NS. OFQ failed to alter the pain threshold at a dose of 0.1 μg but induced decreases of pain threshold at doses of 0.5, 1, and 10 μg. The maximal decrease of pain threshold occurred at 30 min after OFQ injection and they were corresponded to 38 ± 8 μA, 55 ± 9 μA, and 73 ± 11 μA ($P < 0.01$, *vs* NS) for doses of 0.5, 1, and 10 μg, respectively. The effect of 0.5 μg OFQ maintained only about 1 h, while that of 1 μg OFQ lasted for longer, which was kept constant and significant for 2 h. OFQ 10 μg elicited a decrease of pain threshold within 30 min. But, 30 min later, the rats showed obvious reduction in muscular tone and difficulty in tail-flicking, making the measurement of pain threshold impossible. OFQ_{1-10} had no effect on the pain threshold (Fig 1).

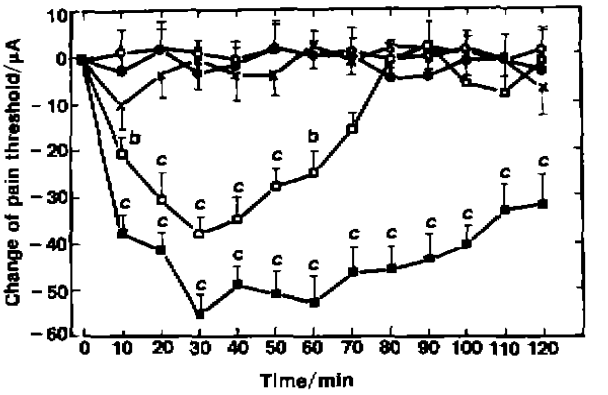

Fig 1. Effect of icv OFQ (0.1 – 1.0 μg) and OFQ_{1-10} on the pain threshold of rat. $\bar{x} \pm s$. $^bP < 0.05$, $^cP < 0.01$ *vs* NS. (○) NS ($n = 4$), (●) OFQ_{1-10} 1.0 μg ($n = 6$), (×) OFQ 0.1 μg ($n = 4$), (□) OFQ 0.5 μg ($n = 4$), (■) OFQ 1.0 μg ($n = 8$).

For comparing the effect of proteinase-inhibitor-protected OFQ with that of unprotected OFQ, the protease inhibitor was not added in the OFQ 1 μg solution in one group ($n = 6$). The effect of unprotected OFQ was similar to that of protected one but maintained for much shorter time (50 min *vs* 120 min of protected-OFQ). The inhibitor alone had no effect on the pain threshold. So, in other sets of experiment, all OFQ solutions were added with the protease inhibitor.

Antagonizing fentanyl-induced analgesia Four groups of rats were icv injected with NS ($n = 4$, as control), Fen (1 μg, $n = 6$), OFQ (0.1 μg) + Fen (1 μg, $n = 4$), and OFQ (1 μg) + Fen (1 μg, $n = 5$). The pain threshold had no obvious change in NS group. Fen induced marked increase of pain threshold over basal level with the maximum of 202 ± 37 μA ($P < 0.01$, *vs* NS) appeared at 30 min after injection and the effect maintained for about 70 min. When OFQ 0.1 or 1 μg was injected together with Fen, the Fen-induced increase of pain threshold was all reduced. The maximal increase was only 75 ± 20 μA and 52 ± 28 μA corresponded to that in OFQ (0.1 μg) + Fen and OFQ (1 μg) + Fen groups ($P < 0.01$, *vs* Fen, Fig 2A).

Attenuating DSLET-induced analgesia Rats were divided into 4 groups of 4 – 8 each and given icv injection of NS (20 μL), DSLET (5 μg), DSLET

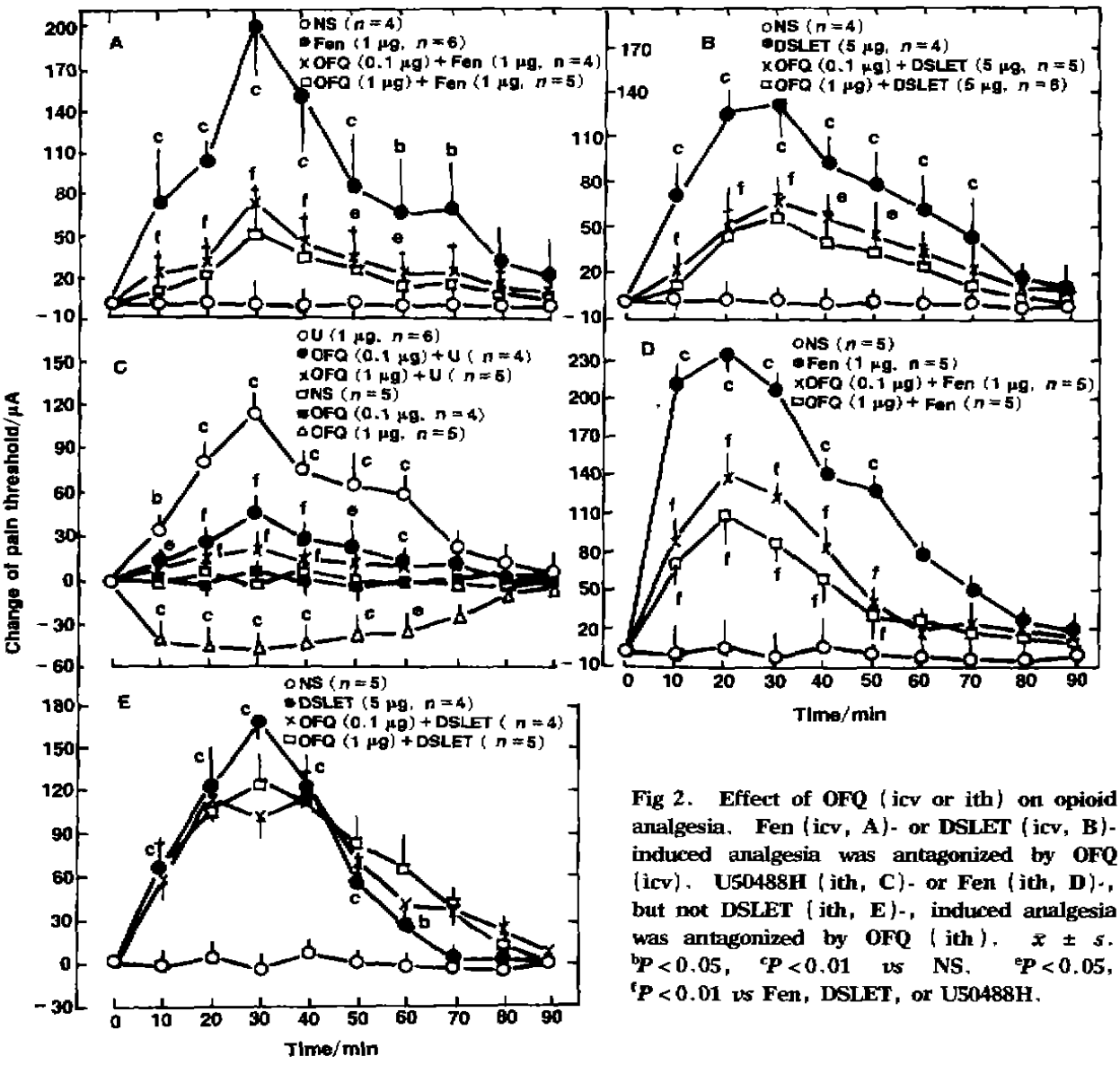

Fig 2. Effect of OFQ (icv or ith) on opioid analgesia. Fen (icv, A)- or DSLET (icv, B)-induced analgesia was antagonized by OFQ (icv). U50488H (ith, C)- or Fen (ith, D)-, but not DSLET (ith, E)-, induced analgesia was antagonized by OFQ (ith). $\bar{x} \pm s$. $^bP<0.05$, $^cP<0.01$ *vs* NS. $^eP<0.05$, $^fP<0.01$ *vs* Fen, DSLET, or U50488H.

(5 μg) + OFQ (0.1 μg), and DSLET (5 μg) + OFQ (1 μg). The pain threshold increased at 10 min following the DSLET injection. The peak value of 133 ± 40 μA occurred at 30 min after injection. When DSLET was combined with 0.1 μg or 1 μg OFQ, the DSLET-induced increase of pain threshold reduced obviously, the maxima of the increase were 67 ± 17 μA and 56 ± 14 μA, respectively ($P<0.01$, *vs* DSLET, Fig 2B).

Effect of the ith injection of OFQ on pain threshold and opioid analgesia

Eliciting decrement of pain threshold and antagonizes U50488H-induced analgesia Rats were ith injected with NS ($n=5$), OFQ (0.1 μg, $n=4$), OFQ (1 μg, $n=5$), U50488H (1 μg, $n=6$), U50488H + OFQ (0.1 μg, $n=4$), and U50488H + OFQ (1 μg, $n=6$). NS and 0.1 μg OFQ had no effect on pain threshold, but 1 μg OFQ induced a marked decrement of pain threshold 10 min after injection with maximum of 46 ± 14 μA. The effect of OFQ maintained constant for 1 h after injection ($P<0.01$, *vs* NS, Fig 2C).

U50488H induced an increase of pain threshold over basal level with maximum of 133 ± 11 μA occurred at 30 min after injection, and the effect was

maintained for 1 h ($P < 0.01$, *vs* NS). When OFQ (0.1 or 1.0 μg) was combined with U50488H, the U50488H-induced increase of pain threshold was reduced ($P < 0.01$, *vs* U50488H, Fig 2C).

Attenuating Fen-induced analgesia Rats (n = 20) were ith injected with NS, Fen (1 μg), Fen + OFQ (0.1 μg), and Fen + OFQ (1 μg), ($n = 5$ in each group). The pain threshold increased markedly at 10 min after the Fen injection and the maximal increase was 238 ± 50 μA ($P < 0.01$, *vs* NS). When Fen was combined with OFQ, whether at 0.1 μg or at 1 μg, OFQ antagonized the Fen-induced increase of pain threshold obviously, which the maximal increases were 152 ± 15 and 142 ± 20 μA in Fen + OFQ (0.1 μg) and Fen + OFQ (1 μg) groups repectively ($P < 0.01$, *vs* Fen, Fig 2D).

No effect on DSLET-induced analgesia Rats were given an ith injection of NS ($n = 5$), DSLET (5 μg, $n = 4$), DSLET + OFQ (0.1 μg, $n = 4$), and DSLET + OFQ (1 μg) ($n = 5$). DSLET induced an analgesic effect ($P < 0.01$, *vs* NS). DSLET-induced analgesia was not much affected by OFQ at both doses ($P > 0.05$, *vs* DSLET, Fig 2E).

DISCUSSION

OFQ elicits hyperalgesia in mice in the first two papers[5-6]. Yet, the followed works have been controversery[12-13]. The present work further demonstrated that OFQ had no effect at small dose (0.1 μg) but had a dose-dependent effect at larger doses (0.5 – 10 μg) in inducing hyper-reaction of rat to noxious electric stimulus in tail-flick model. At dose of 1 μg (0.55 nmol), OFQ showed the best effect without affecting the muscular tone; while at the dose of 10 μg (5.5 nmol), OFQ resulted in obvious reduction of muscular tone and difficulty in tail-flicking. It was also found that the effect of OFQ protected by the proteinase inhibitor was better than unprotected OFQ. As the inhibitor is a serine protease inhibitor capable of inhibiting trypsin, chymotrypsin as well as kallikrein, the degradation of OFQ *in vivo* may also be caused by these like enzymes. This result hints that the activity of OFQ fragment may reduced. The result that OFQ_{1-10} has no effect on pain threshold further demonstrates that the ten amino residues in C-terminal has no activity and the whole sequence of OFQ may be essential to its activity[14]. Yet, the relationship between the activity and conformation of OFQ need to be further studied.

It is an interesting issue that the action of OFQ is different from the analgesic effect of opioid peptide though they share the similarity in structures. Present study further revealed that the icv injection of OFQ significantly antagonized opioid analgesia mediated by μ- and δ-opioid receptor in the brain; and the ith injection of OFQ also obviously antagonized opioid analgesia mediated by μ- and κ- but not δ-opioid receptors in the spinal cord. Our recent work showed that OFQ significantly reduced acupuncture analgesia which played its analgesic role mainly via activating the endogenous opioid peptide[15]. These results indicated that OFQ had an anti-opioid effect in the central nervous system which may account for the hyperalgesic effect of OFQ: the inhibition of endogenous opioid peptide on pain transmission may be antagonized by OFQ, hence the animals showed hyperactivity in response to noxious stimulus. However, why the ith injection of OFQ has no effect on δ-opioid receptor mediated analgesia remains unclear and the mechanism underlying the antagonistic interaction between OFQ and opioids is yet to be clarified.

In conclusion, the newly discovered OFQ has no effect on nociception at small dose but has a dose-dependent effect in eliciting hyperalgesia at larger doses, and has an anti-opioid effect which may account for the hyperalgesic action.

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10-14

孤啡肽(orphanin FQ)对大鼠阿片镇痛的拮抗作用[1]

R971.2

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关键词 孤啡肽; 疼痛感受器; 阿片镇痛药; μ阿片受体; δ阿片受体; κ阿片受体

目的: 研究孤啡肽(OFQ)对痛与阿片镇痛的影响. **方法**: 脑室(icv)与鞘内(ith)给药, 以大鼠甩尾模型测痛. **结果**: 小剂量 OFQ (0.1 μg) icv 及 ith 给药对痛反应均无影响; 较大剂量 OFQ (0.5 – 10 μg)可使痛反应增强. OFQ_{1-10}(OFQ 的一个片段) icv 对痛反应无影响. μ-受体激动剂芬太尼 (1 μg)、δ-激动剂 DSLET (5 μg) icv 或 ith 给药, 以及 κ-激动剂 U50488H (1 μg) ith 给药, 可使痛阈明显增加. 0.1 μg 或 1 μg OFQ 与上述药物合用后, 痛阈增加明显减少(除鞘内与 DSLET 合用外). **结论**: OFQ 可增强大鼠的痛反应, 在脑内对抗由 μ-和 δ-受体介导的阿片镇痛, 在脊髓对抗由 κ-和 μ-但不是由 δ-受体介导的镇痛.