

ORPHANIN FQ or nociceptin (OFQ/N<sub>1-17</sub>) is a recently discovered peptide which, upon intracerebroventricular administration, reverses opioid-mediated analgesias. OFQ/N<sub>1-17</sub> terminals are located in the periaqueductal gray (PAG), a structure known to be involved in pain modulation, suggesting that the functional anti-opioid effects of OFQ/N<sub>1-17</sub> are mediated by PAG neurons. To test this, subsequent microinjections of morphine or kainic acid and OFQ/N<sub>1-17</sub> were made into the PAG of awake rats. Administration of OFQ/N<sub>1-17</sub> attenuated the tail flick inhibition produced by both morphine and kainic acid microinjection. OFQ/N<sub>1-17</sub> attenuation of antinociception produced by a neuroexcitant indicates that OFQ/N<sub>1-17</sub> reverses opioid antinociception by inhibiting PAG output neurons.

## Antinociception mediated by the periaqueductal gray is attenuated by orphanin FQ

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### Introduction

Ligands that bind to opioid receptors, such as morphine, are the most effective treatment available for pain. The discovery of a G protein-coupled receptor<sup>1,2</sup> that is homologous to  $\mu$ ,  $\delta$  and  $\kappa$  opioid receptors, but is not bound by classic opioid peptides suggested the existence of a previously unknown endogenous opioid. The ligand for this orphan opioid-like receptor was found to be a 17 amino acid peptide called orphanin FQ<sup>3</sup> or nociceptin<sup>4</sup> (OFQ/N<sub>1-17</sub>). Despite similarities between OFQ/N<sub>1-17</sub> and opioid peptides, intracerebroventricular (i.c.v.) administration of OFQ/N<sub>1-17</sub> to mice does not produce analgesia like traditional opioids, but was reported to enhance nociception.<sup>3,4</sup> Subsequently, Mogil and colleagues<sup>5</sup> demonstrated that this apparent hyperalgesia was actually an attenuation of stress-induced analgesia (SIA) that resulted from the i.c.v. injection procedure. In addition to attenuating SIA, i.c.v. administration of OFQ/N<sub>1-17</sub> has been shown to attenuate the antinociceptive effect of i.c.v. administration of  $\mu$ ,  $\delta$  and  $\kappa$  agonists and systemic administration of morphine.<sup>5-7</sup>

Given that i.c.v. administration of OFQ/N<sub>1-17</sub> could act at a number of brain sites, the brain structure(s) via which OFQ/N<sub>1-17</sub> attenuates antinociception is not known. However, a likely candidate is the

periaqueductal gray (PAG). The PAG is known to be involved in the modulation of nociception<sup>8</sup> and OFQ/N<sub>1-17</sub> terminals and receptors have been reported in the PAG.<sup>9,10</sup> In addition, OFQ/N<sub>1-17</sub> has been shown to inhibit PAG neurons recorded *in vitro*.<sup>11</sup> Surprisingly, this inhibitory effect is identical to that produced by direct application of morphine, except that OFQ/N<sub>1-17</sub> inhibits nearly all PAG neurons<sup>11</sup> whereas morphine only inhibits a subset of PAG neurons.<sup>12</sup> Even though both morphine and OFQ/N<sub>1-17</sub> inhibit PAG neurons, these compounds could produce opposite effects on nociception depending on which neurons are affected. If this is the case, then administration of OFQ/N<sub>1-17</sub> should attenuate the antinociception produced by direct activation of PAG output neurons as occurs with microinjection of excitatory amino acids. Thus, the two objectives of the present study were to determine whether the ventral aspect of the PAG is a site at which OFQ/N<sub>1-17</sub> can block the antinociceptive effects of morphine, and if so, whether this can be explained by inhibition of PAG output neurons.

### Materials and Methods

**Animals:** Thirty-two male Sprague–Dawley rats (300–350 g) were anesthetized with pentobarbital (55 mg/kg, i.p.) and stereotaxically implanted with a

25 gauge guide cannula (12 mm long) aimed at the ventral PAG. The guide cannula was affixed to two screws in the skull with dental cement. Following surgery, a stylet was inserted into the guide cannula and the rat allowed at least 1 week to recover before testing. Rats were housed individually in a temperature controlled room with a 12:12 h reversed light cycle. Rats were habituated to the following: handling, restraint in a Plexiglas tube, and the injection procedure prior to testing.

**Microinjection procedure:** Drug microinjections were made through a 31 gauge injection cannula that extended 2 mm beyond the tip of the guide cannula. All injections were made in a volume of 0.4  $\mu$ l over 40 s while the rat was gently restrained. The injection cannula remained in place for an additional 20 s to minimize backflow of the drug up the cannula track. OFQ/N<sub>1-17</sub> was purchased from Phoenix Pharmaceutical Company as a lyophilized powder. Immediately prior to use, the peptide was dissolved in artificial CSF (aCSF) and kept on ice until used.

**Experiment 1:** On the test day, baseline nociception was assessed using the tail flick reflex to 52°C water. Briefly, the caudal third of the tail was placed in the water and the latency to move the tail measured. Immediately following two baseline tail flick tests, morphine (5  $\mu$ g) was injected into the ventral PAG. Thirty minutes later the latency for the tail flick reflex was assessed, followed immediately by microinjection of OFQ/N<sub>1-17</sub> (10 nmol) or aCSF into the same PAG injection site. Tail flick latency was measured 15 and 30 min later.

**Experiment 2:** The procedure for this experiment was identical to Experiment 1 except that antinociception was produced by microinjecting kainic acid (40 pmol), instead of morphine into the ventral PAG. Tail flick latency was measured 2, 5 and 15 min after kainic acid was injected. OFQ/N<sub>1-17</sub> or aCSF was microinjected immediately following the 2 min test.

**Histology:** Following testing, rats were given a lethal injection of pentobarbital (120 mg/kg, i.p.). The injection site was marked by injecting cresyl violet (0.3  $\mu$ l) into the same site as the drugs were administered previously. The brain was removed and placed in formalin. At least 1 week later brain sections were cut at 50  $\mu$ m, placed on slides, and viewed under a microscope for localization of the injection site.

**Data analysis:** To determine whether OFQ/N<sub>1-17</sub> reversed antinociception, only rats with a tail flick latency of  $\geq$  6.5 s following morphine or kainic acid administration were included in data analysis (base-

line latencies ranged from 2.0 to 4.1 s). Differences in mean tail flick latency between OFQ/N<sub>1-17</sub>-treated and aCSF-treated rats were analyzed with a *t*-test for independent means.

## Results

**Experiment 1:** Microinjection of morphine inhibited the tail flick reflex in 10 of 17 rats. Injection sites were located in or immediately adjacent to the vPAG (Fig. 1). In these 10 rats, mean baseline tail flick latency rose from  $3.2 \pm 0.2$  to  $9.9 \pm 0.3$  s 30 min following microinjection of morphine. Subsequent administration of OFQ/N<sub>1-17</sub> into the PAG produced a significant reduction in tail flick latency compared with rats receiving a microinjection of aCSF ( $t(8) = 5.543$ ,  $p < 0.05$ ). The difference in tail flick latency between OFQ/N<sub>1-17</sub>-treated and aCSF-treated rats was no longer evident 30 min after these compounds were injected (Fig. 2).

**Experiment 2:** Microinjection of kainic acid inhibited the tail flick reflex in 9 of 15 rats tested. Injection sites were located in or immediately adjacent to the ventral PAG (Fig. 1). Mean baseline tail flick latency rose from  $2.9 \pm 0.2$  to  $9.2 \pm 1.4$  s immediately following administration of kainic acid. Subsequent microinjection of OFQ/N<sub>1-17</sub> caused a significant decrease in tail flick latency compared to rats receiving a microinjection of aCSF ( $t(7) = 2.472$ ;  $p < 0.05$ ). The antinociceptive effect of kainic acid was no longer evident 15 min after administration (Fig. 3).

## Discussion

These data demonstrate that microinjection of OFQ/N<sub>1-17</sub> into the ventral PAG attenuates antinociception mediated by the PAG. This finding is consistent with the anatomical localization of OFQ/N<sub>1-17</sub> terminals and receptors in the PAG.<sup>9,10</sup> Moreover, our data suggest that the ability of i.c.v. OFQ/N<sub>1-17</sub> to block opioid antinociception may be mediated, at least in part, by PAG neurons.

Given that morphine and kainic acid activate PAG output neurons through different mechanisms, examining the effects of OFQ/N<sub>1-17</sub> on the antinociception produced by these two compounds provides insight into the anti-opioid action of OFQ/N<sub>1-17</sub>. The direct effect of local administration of opioids is to inhibit a subset of PAG neurons<sup>12</sup> which appears to disinhibit PAG output neurons.<sup>13,14</sup> In contrast, excitatory amino acid agonists such as kainic acid excite PAG output neurons directly. The fact that microinjection of OFQ/N<sub>1-17</sub> into the PAG inhibited the antinociceptive effect of both morphine and kainic

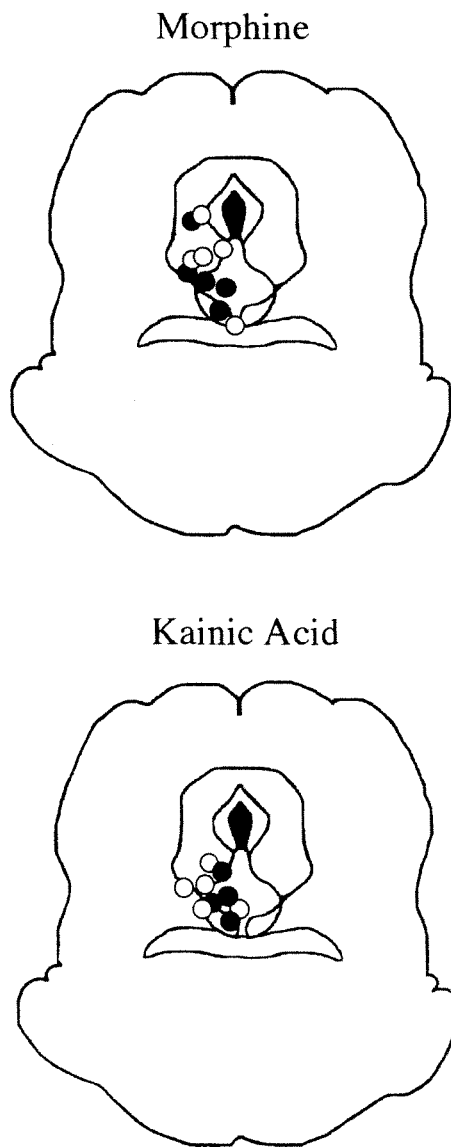


FIG. 1. Location of microinjection sites for morphine (top) and kainic acid (bottom). Half of the morphine-treated and half of the kainic acid-treated rats received a subsequent injection of OFQ/N<sub>1-17</sub> (closed circles), while the other half were injected with artificial CSF (open circles).

acid indicates that OFQ/N<sub>1-17</sub> inhibits PAG output neurons. Such a mechanism is consistent with a recent report showing that administration of OFQ/N<sub>1-17</sub> to a PAG tissue slice produces inhibition of nearly every ventral PAG neuron studied.<sup>11</sup>

The present data indicate that the anti-analgesic effect of OFQ/N<sub>1-17</sub> occurs by inhibition of PAG output neurons that receive input from opioid sensitive neurons. Because both OFQ/N<sub>1-17</sub> and morphine inhibit a subset of PAG neurons,<sup>11,12</sup> the only way that OFQ/N<sub>1-17</sub> can modulate the effects of exogenous opioids is to reverse the effects of opioids on neurons downstream from opioid sensitive neurons. Thus, OFQ/N<sub>1-17</sub> appears to inhibit PAG output

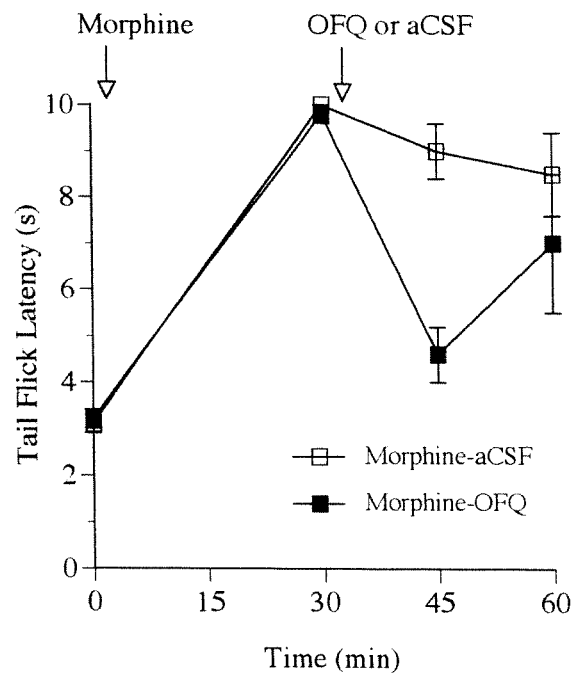


FIG. 2. Change in tail flick latency as a result of subsequent injections of morphine (5  $\mu$ g/0.5  $\mu$ l) and OFQ/N<sub>1-17</sub> (10 nmol) or aCSF into the ventral PAG. Microinjection of OFQ/N<sub>1-17</sub> reversed the antinociceptive effect of morphine administration compared with aCSF-treated rats ( $p < 0.05$ ).

neurons that are activated by opioid administration. Given that ventral PAG output neurons project to the rostral ventromedial medulla which in turn project to the spinal dorsal horn to modulate nociception,<sup>15</sup> inhibition of PAG output neurons would be an effective means of reversing antinociception.

Although OFQ/N<sub>1-17</sub> can function as an anti-opioid peptide within the PAG, OFQ/N<sub>1-17</sub> may have other effects in other regions of the nervous system. OFQ/N<sub>1-17</sub> and its receptor are widely distributed in the CNS, and OFQ/N<sub>1-17</sub> has been shown to inhibit neurons at numerous sites. For example, OFQ/N<sub>1-17</sub> inhibits neurons in the dorsal horn,<sup>16,17</sup> rostral ventromedial medulla,<sup>18</sup> locus coeruleus,<sup>19</sup> hippocampus<sup>20</sup> and cerebral cortex.<sup>21</sup> Such widespread inhibitory effects indicate that this peptide has a range of roles in nervous system function, and may explain why intrathecal administration of OFQ/N<sub>1-17</sub> inhibits nociception,<sup>7,17</sup> whereas supraspinal administration of OFQ/N<sub>1-17</sub> inhibits antinociception.<sup>22</sup> These seemingly contradictory effects are best explained by the fact that OFQ/N<sub>1-17</sub> is able to inhibit many classes of neurons.

## Conclusion

The discovery of the orphan opioid-like receptor and its endogenous ligand OFQ/N<sub>1-17</sub> has generated

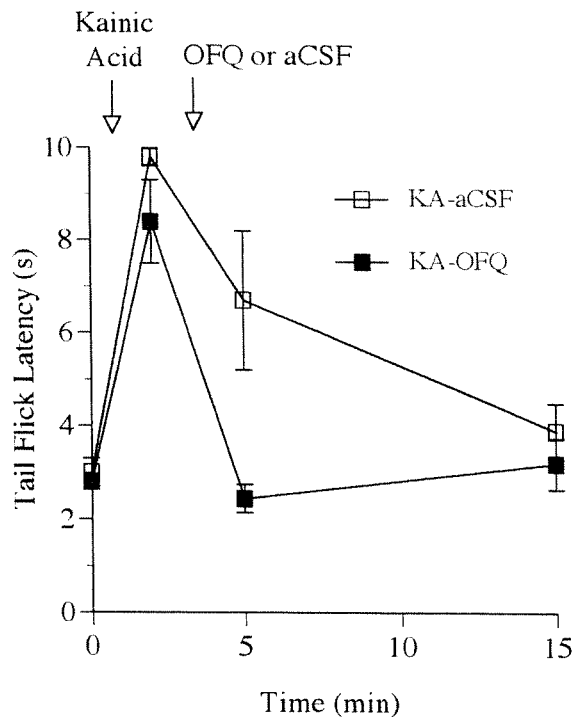


FIG. 3: Change in tail flick latency as a result of subsequent injections of kainic acid (KA; 40 pmol) and OFQ/N<sub>1-17</sub> (10 nmol) or aCSF into the ventral PAG. Microinjection of OFQ/N<sub>1-17</sub> reversed the antinociceptive effect of kainic acid administration compared with aCSF-treated rats ( $p < 0.05$ ).

much speculation about its functional role. Although the similarities between OFQ/N<sub>1-17</sub> and other opioid peptides suggests that OFQ/N<sub>1-17</sub> may be a new antinociceptive agent, behavioral studies suggest the opposite – that OFQ/N<sub>1-17</sub> is an anti-opioid peptide.

Our findings extend the results of previous behavioral studies by demonstrating that the anti-opioid effects of OFQ/N<sub>1-17</sub> are mediated, at least in part, by inhibition of PAG output neurons.

## References

- Bunzow JR, Saex C, Mortrud M et al. *FEBS Lett* **347**, 284–288 (1994).
- Mollereau C, Parmentier M, Mailleux P et al. *FEBS Lett* **341**, 33–38 (1994).
- Reinscheid RK, Nothacker HP, Bourson A et al. *Science* **270**, 792–794 (1995).
- Meunier JC, Mollereau C, Toll L et al. *Nature* **377**, 532–535 (1995).
- Mogil JS, Grisel JE, Reinscheid RK et al. *Neuroscience* **75**, 333–337 (1996).
- Mogil JS, Grisel JE, Zhangs G et al. *Neurosci Lett* **214**, 131–134 (1996).
- Tian JH, Xu W, Fang Y et al. *Br J Pharmacol* **120**, 676–680 (1997).
- Morgan MM. Differences in antinociception evoked from dorsal and ventral regions of the caudal periaqueductal gray matter. In: Depaulis A and Bandler R, eds. *The Midbrain Periaqueductal Gray Matter*. New York: Plenum Press, 1991: 139–150.
- Anton B, Fein J, To T et al. *J Comp Neurol* **368**, 229–251 (1996).
- Schulz S, Schreff M, Nuss D et al. *Neuroreport* **7**, 3021–3025 (1996).
- Vaughan CW, Ingram SL and Christie MJ. *J Neurosci* **17**, 996–1003 (1997).
- Osborne PB, Vaughan CW, Wilson HI and Christie MJ. *J Physiol (Lond)* **490**, 383–389 (1996).
- Depaulis A, Morgan MM and Liebeskind JC. *Brain Res* **436**, 223–228 (1987).
- Moreau JL and Fields HL. *Brain Res* **397**, 37–46 (1986).
- Basbaum AI and Fields HL. *Annu Rev Neurosci* **7**, 309–38 (1984).
- Wang XM, Zhang KM and Mokha SS. *J Neurophysiol* **76**, 3568–3572 (1996).
- Stanfa LC, Chapman V, Kerr N and Dickenson AH. *Br J Pharmacol* **118**, 1875–1877 (1996).
- Heinricher MM, McGaraughty S and Grandy DK. *Soc Neurosci Abstr* **22**, 1368 (1996).
- Connor M, Vaughan CW, Chieng B and Christie MJ. *Br J Pharmacol* **119**, 1614–1618 (1996).
- Yu TP, Fein J, Phan T et al. *Hippocampus* **7**, 88–94 (1997).
- Nicol B, Lambert DG, Rowbotham DJ et al. *Br J Pharmacol* **119**, 1081–1083 (1996).
- Grisel JE, Mogil JS, Belknap JK and Grandy DK. *NeuroReport* **7**, 2125–2129 (1996).

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