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[Phe$^1\Psi$(CH$_2$-NH)Gly^2]nociceptin-(1-13)-NH$_2$ acts as an agonist of the orphanin FQ/nociceptin receptor in vivo

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Abstract

The orphanin FQ/nociceptin (OFQ/N) derivative peptide, [Phe$^1\Psi$(CH$_2$-NH)Gly^2] nociceptin-(1-13)-NH$_2$ (PheΨ), has been claimed to be both an antagonist and an agonist of the orphan opioid receptor (ORL1) in different in vitro assays. We now report the dose-dependent inhibition of morphine analgesia by PheΨ in mice, an effect parallel to that of OFQ/N. Further, the anti-opioid actions of OFQ/N are not blocked by PheΨ. Thus, PheΨ acts as an ORL1 receptor agonist, not an antagonist, in vivo.

Keywords: Anti-opioid; ORL1 receptor; Morphine

The endogenous ligand of the orphan opioid receptor, ORL1, which bears substantial structural homology to the previously cloned μ-, δ-, and κ-opioid receptors, was recently isolated by two separate groups and named orphanin FQ (Reinscheid et al., 1995) and nociceptin (Meunier et al., 1995). The role of orphanin FQ/nociceptin (OFQ/N) in pain modulation remains quite controversial, with various laboratories reporting no effect, hyperalgesic, analgesic, and/or anti-analgesic effects after supraspinal injection in rodents (see Henderson and McKnight, 1997). Decisive confirmation of OFQ/N's true role has awaited the isolation of a specific ORL1 receptor antagonist. Recently, Guerrini et al. (1998) proposed the OFQ/N derivative, [Phe$^1\Psi$(CH$_2$-NH)Gly^2]nociceptin-(1-13)-NH$_2$ (hereinafter called PheΨ), as an ORL1-specific receptor antagonist based on its actions vis-à-vis OFQ/N in the guinea pig ileum and mouse vas deferens assays. The notion of PheΨ as a selective ORL1 receptor antagonist has been more recently disputed by Butour et al. (1998), who provide evidence that PheΨ is a pure and potent agonist at ORL1 receptors expressed by transformed Chinese hamster ovary cells.

The intent of the present study was to determine whether PheΨ is an ORL1 receptor agonist or antagonist in an in vivo assay. We used the well-documented and uncontroversial nociception-related paradigm: reversal of supraspinal morphine analgesia by OFQ/N in mice (Grisel et al., 1996; Mogil et al., 1996). Morphine analgesia was evaluated on the 50°C tail-immersion/withdrawal assay in the presence of varying doses of PheΨ, either alone or in combination with OFQ/N.

Adult male and female Swiss–Webster mice were obtained from Simonsen. Mice were group housed in a reversed 12:12 h light cycle and a constant ambient temperature of 22 ± 2°C. PheΨ was obtained from Tocris and OFQ/N was obtained from NIDA/NIH; both were diluted in artificial cerebrospinal fluid (aCSF). Morphine sulfate was obtained from Sigma and dissolved in physiological saline. Nociceptive sensitivity was measured as latency to elicit a reflexive withdrawal after immersion of the distal half of the tail in a 50.0 ± 0.5°C water bath. During testing, subjects were lightly restrained in cloth pockets which they voluntarily entered. An experienced observer performed two measurements at each time point, which were averaged. A maximum latency time ('cut-off') was set at 12 s.

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On the day of testing, subjects were weighed and acclimatized to the experimental room for a minimum of 30 min. Following baseline nociceptive assessment, 12.5 mg/kg morphine or saline (10 ml/kg) was injected intraperitoneally (i.p.). A second 'baseline' measurement was taken 15 min post i.p. injection to verify the existence of morphine analgesia. Subjects were then briefly anesthetized with isoflurane/oxygen, and intracerebroventricular (i.c.v.) injections were administered via a 10 μl Hamilton microsyringe into the lateral ventricle according to the method of Laursen and Belknap (1986). All i.c.v. injections were given in a 2.5 μl volume, and consisted of: 0, 0.3, 3, 10, or 60 nmol PheΨ, 5 nmol OFQ/N, or 5 nmol OFQ/N + 10 nmol PheΨ ($n = 5–10$ per condition). With

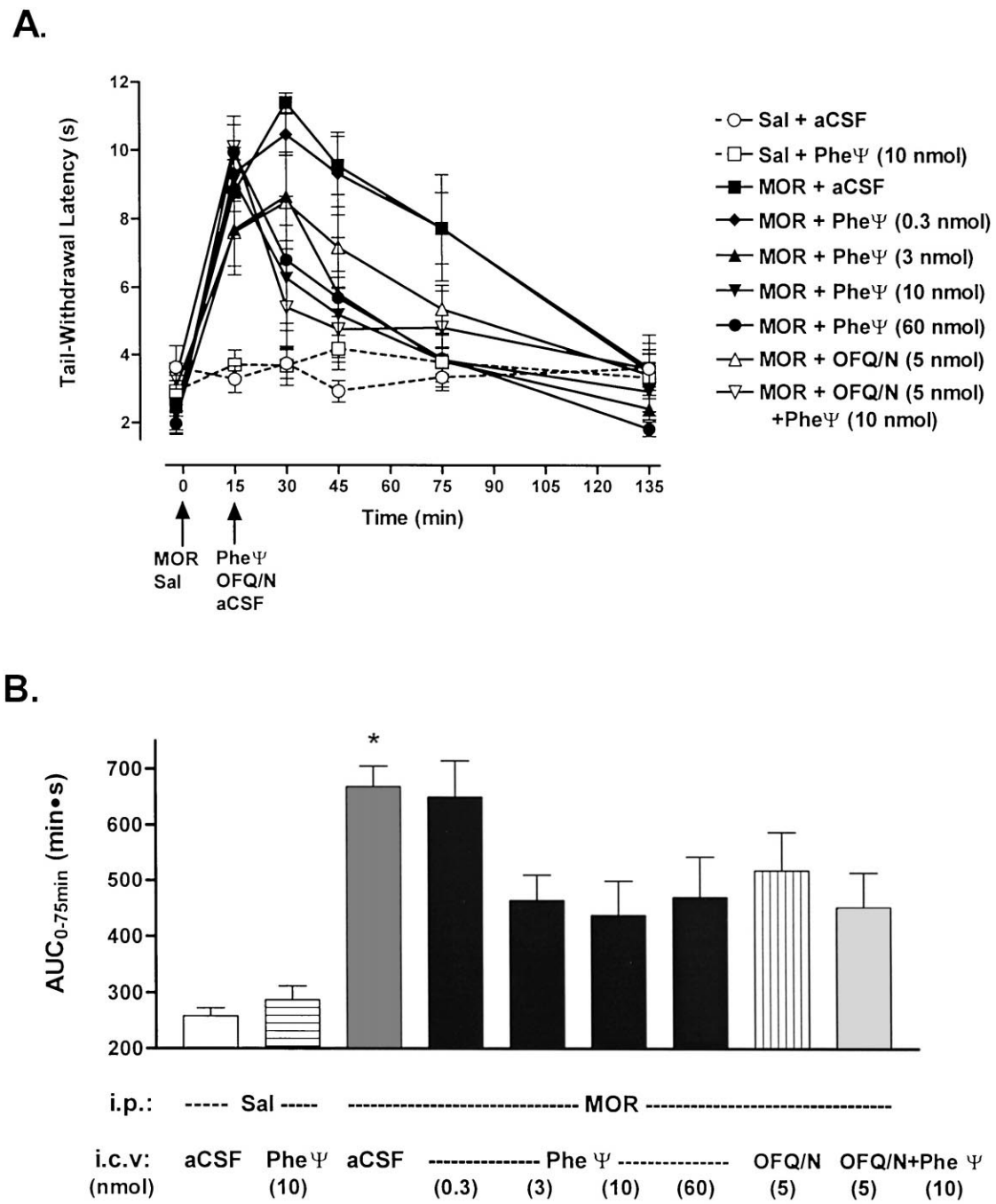

Fig. 1. Reversal of morphine analgesia by PheΨ and OFQ/N. Following assessment of baseline nociception on the 50°C tail-withdrawal test, mice were injected systemically with morphine (MOR; 12.5 mg/kg; solid lines) or saline (Sal; 10 ml/kg, i.p.; broken lines). After retesting at 15 min, all mice received an i.c.v. injection of PheΨ (0.3–60 nmol) and/or OFQ/N (5 nmol), or aCSF (2.5 μl), and were retested 15, 30, 60 and 120 min later. Mean tail-withdrawal latencies (±S.E.M.) are shown in (A); integrated values from 0 to 75 min ($AUC_{0-75\ min}$; based on the known duration of action of peptides like PheΨ and OFQ/N) are presented in (B). *Significantly different from all groups except MOR + PheΨ (0.3 nmol).

the exception of the latter group (that had been previously i.c.v. injected), all mice were experimentally naive. Nociceptive sensitivity was then assessed at 15, 30, 60, and 120 min post i.c.v. injection. Overall analgesia was expressed as area under the time x latency curve (AUC; 0–75 min) using the trapezoidal rule.

As the design was unbalanced, a one-way ANOVA was used to compare AUC means from all groups, and revealed a significant main effect of condition ($F_{8,49} = 7.04$, $P < 0.001$). Individual group comparisons were made using Fisher's LSD post hoc test and an α level of 0.05. As shown in Fig. 1, whereas 10 nmol PheΨ does not significantly alter latencies by itself, this compound dose-dependently attenuates morphine analgesia. Significant effects were obtained using 3 nmol PheΨ, and doses ≥ 3 nmol produced asymptotic but incomplete reversal of the analgesia produced by this dose of morphine. As predicted from our previous work, 5 nmol OFQ produced a significant but incomplete reversal of morphine analgesia, and this effect was neither significantly reversed nor enhanced by co-administration of 10 nmol PheΨ.

These in vivo results support the in vitro findings of Butour et al. (1998) that PheΨ is in fact not an ORL1 receptor antagonist, but rather an agonist. The potency of PheΨ appears to compare well with that of OFQ/N; although PheΨ was incompletely efficacious against morphine analgesia, a similar limitation of OFQ/N has been described (Grisel et al., 1996). The delineation of the true role of the ORL1 receptor in pain modulation thus still awaits the development of an appropriate pharmacological tool.

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