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Supraspinal hyperalgesia and spinal analgesia by [Phe$^1\psi$(CH$_2$-NH)Gly^2]nociceptin-(1–13)-NH$_2$ in rat

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Abstract

[Phe$^1\psi$(CH$_2$-NH)Gly^2]nociceptin-(1–13)-NH$_2$, a pseudopeptide analog of nociceptin, was originally seen as an antagonist of nociceptin receptors. In the present study, it was observed that intracerebroventricular (i.c.v.) injection of this pseudopeptide (1, 5, 10 μg) significantly decreased the tail-flick latency of rats, indicating a hyperalgesic effect, while intrathecal (i.t.) injection of it (1, 2.5, 10 μg) dramatically increased the tail-flick latency, indicating an analgesic effect. This strengthened the in vivo evidence that [Phe$^1\psi$(CH$_2$-NH)Gly^2]nociceptin-(1–13)-NH$_2$ might be an agonist of nociceptin receptors.

Keywords: Nociceptin pseudopeptide analog; Hyperalgesia; Analgesia

Nociceptin (also known as orphanin FQ), a newly discovered heptadecapeptide, is a potent agonist for nociceptin receptors (Meunier et al., 1995; Reinscheid et al., 1995). It has been proved to produce numerous effects, including supraspinal hyperalgesia, spinal analgesia, motor impairment, suppression of spatial learning and stimulation of food intake, etc. (Meunier, 1997). Since a specific antagonist was not available, it could not be determined whether these effects of nociceptin might be mediated by nociceptin receptors. Recently, the pseudopeptide, [Phe$^1\psi$(CH$_2$-NH)Gly^2]nociceptin-(1–13)-NH$_2$, an analog of nociceptin, was reported to prevent the inhibition of electrically induced contraction of guinea pig ileum and mouse vas deferens by nociceptin (Guerrini et al., 1998) and was thus identified as a new selective antagonist of the nociceptin receptor. However, the following studies have yielded different results. It was reported that intrathecal [Phe$^1\psi$(CH$_2$-NH)Gly^2]nociceptin-(1–13)-NH$_2$ produced a dose-dependent depression of the nociceptive reflex in rat and this effect was similar to that of nociceptin. So, it was proposed that this pseudopeptide is an agonist of nociceptin receptors (Xu et al., 1998). Others observed a potent and fully efficacious inhibiting effect of the pseudopeptide on forskolin-induced accumulation of cAMP in transformed Chinese hamster ovary (CHO) cells expressing the nociceptin receptor, which also indicated a nociceptin-like action of the pseudopeptide (Butour et al., 1998). These findings prompted us to test the supraspinal and spinal effects of the pseudopeptide on tail flick latency of rats to obtain in vivo data for determining its pharmacologic characteristics.

The present study was conducted with male Sprague–Dawley rats weighing 180–240 g (Experimental Animal Center, Shanghai Medical University, China), and was approved by the local research ethic committee. [Phe$^1\psi$-(CH$_2$-NH)Gly^2]nociceptin-(1–13)-NH$_2$ was purchased from Phoenix Pharmaceuticals (CA, USA). According to the manufacturer's data, the pseudopeptide was 99% pure and had the expected molecular weight. Before the experiments, a stainless steel injection cannula (o.d. 0.8 mm) was implanted stereotaxically into the right lateral ventricle of the rat brain under anesthesia with sodium pentobarbital (30 mg/kg, i.p.). In another group of rats, a PE-10 catheter (o.d. 0.61 mm) was put into the subarachnoid space at the level of the cauda equina and then was carefully inserted rostrally, with its tip aimed at the lumbar enlargement of the spinal cord. Experiments were performed at room temperature (22 ± 1°C) 72–96 h after surgery. The noci-

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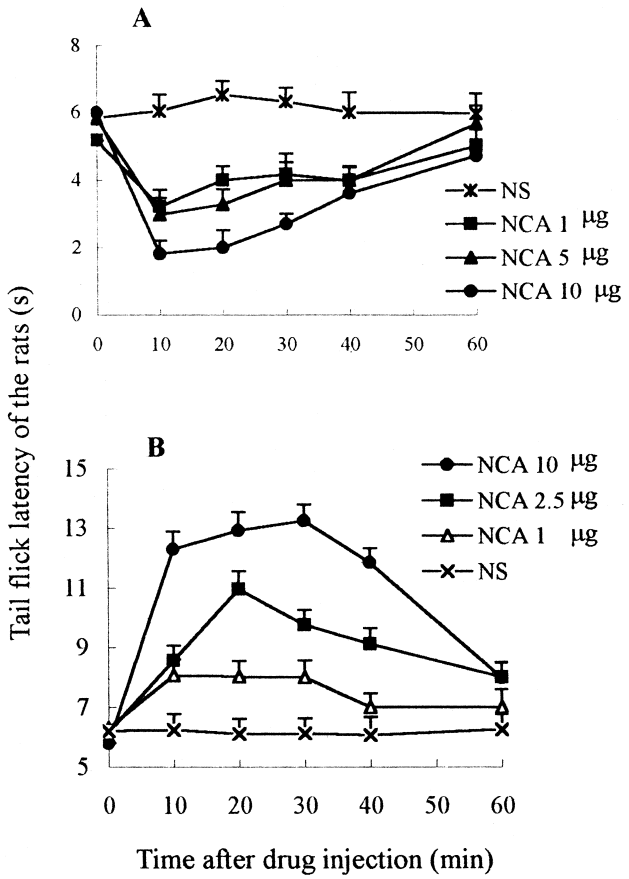

Fig. 1. Effect of i.c.v. (A) or i.t. (B) injection of [Phe$^1\psi$(CH$_2$-NH)Gly^2]nociceptin-(1–13)-NH$_2$ (NCA) on tail-flick latency of rats. Note the dose-dependent decrease (A) or increase (B) of the tail flick latency. $P < 0.05$ vs. normal saline. Each value is the mean ± S.E.M. $N = 9$ for each group.

ceptive test was the tail-flick assay. The rats were lightly restrained in a wooden holder. The distal part of the tail was immersed in 50°C water and the tail-flick latency was measured. Before drug injection, each rat was tested to obtain the baseline latency (ranged from 5 to 7 s, and 15 s was the cut-off time). Then i.c.v. (in 3 min) or i.t. (in 10 s) injection was performed separately. The pseudopeptide was dissolved in sterilized normal saline and injected via the catheter in a volume of 10 μl and followed by 10 μl normal saline for flushing. Each rat was tested at 10, 20, 30, 40, and 60 min after drug injection. After the experiments, the rats were killed and the location of drug injection was verified. Data were expressed as the means ± S.E.M. and the significance of differences was assessed by analysis of variance (ANOVA) and *Q*-test.

Fig. 1A shows that i.c.v. injection of [Phe$^1\psi$(CH$_2$-NH)Gly^2]nociceptin-(1–13)-NH$_2$ (1, 5, 10 μg) dose dependently decreased the tail flick latency, and the effect lasted for more than 40 min ($P < 0.05$ vs. normal saline). Our data indicated a long-lasting supraspinal hyperalgesic effect by the pseudopeptide.

Fig. 1B shows that i.t. injection of [Phe$^1\psi$(CH$_2$-NH)Gly^2]nociceptin-(1–13)-NH$_2$ (1, 2.5, 10 μg) increased the tail flick latency of rats and that, at higher doses (2.5 or 10 μg) the effect lasted for more than 40 min, indicating a potent and long-lasting analgesia by the pseudopeptide in rat spinal cord.

Thus, our in vivo experiments suggested that [Phe$^1\psi$-(CH$_2$-NH)Gly^2]nociceptin-(1–13)-NH$_2$ has an agonist action on the nociceptin receptor. In the present study, the supraspinal hyperalgesia and spinal analgesia by the pseudopeptide were similar to the effect of nociceptin (Meunier, 1997), except that the former effect was longer than the latter. In the present experiments, both the supraspinal and the spinal effect of the pseudopeptide lasted more than 40 min, while in our previous study, nociceptin produced a short-lasting supraspinal hyperalgesia (no more than 20 min, Wang et al., in press). Tian et al. (1997) had reported a shorter spinal analgesia (20 min) produced by nociceptin compared to that by the pseudopeptide. Since [Phe$^1\psi$-(CH$_2$-NH)Gly^2]nociceptin-(1–13)-NH$_2$ was introduced to protect the peptides from degradation by aminopeptidases, it was proposed that it may have a long duration of action in in vivo experiments (Guerrini et al., 1998). This was confirmed again by our data.

Indeed, the discrepancy between the antagonistic effect of the pseudopeptide on nociceptin receptor in smooth muscle and the agonistic effect of it in in vivo tests is yet to be clarified. However, our study strengthened the accumulating evidence that [Phe$^1\psi$(CH$_2$-NH)Gly^2]nociceptin-(1–13)-NH$_2$ might be an agonist of nociceptin receptors.

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