British Journal of Pharmacology (1998) 125, 949–952
http://www.stockton-press.co.uk/bjp

SPECIAL REPORT

Evidence that [Phe^1 ψ(CH_2-NH)Gly^2]nociceptin-(1-13)-NH_2, a peripheral ORL-1 receptor antagonist, acts as an agonist in the rat spinal cord

[1,2]Kate J. Carpenter & [1]Anthony H. Dickenson

[1]Department of Pharmacology, University College London, Gower Street, London WC1E 6BT

[Phe^1 ψ(CH_2-NH)Gly^2]nociceptin-(1-13)-NH_2, a pseudopeptide analogue of nociceptin is an antagonist in peripheral assays. Here, using *in vivo* electrophysiological recordings of dorsal horn neurones, [Phe^1 ψ(CH_2-NH)Gly^2]nociceptin-(1-13)-NH_2 appears to have agonist activity after spinal administration. The noxious evoked activity of the neurones was inhibited by [Phe^1 ψ(CH_2-NH)Gly^2]nociceptin-(1-13)-NH_2, which was as potent as nociceptin itself.

Keywords: nociceptin; spinal cord; ORL-1 receptor; antinociception

Introduction Nociceptin (orphanin FQ), the endogenous ligand for the opioid receptor-like orphan receptor (ORL-1), is implicated in many biological processes including pain (see Meunier, 1997 for review). The ORL-1 transcript (Wick *et al*., 1994) and ORL-1-like immunoreactivity (Anton *et al*., 1996) have been detected in rat spinal cord and human central nervous system (CNS) (Peluso *et al*., 1998). Williams *et al*. (1998) have demonstrated basal and an electrically evoked release of nociceptin-like material from the rat dorsal horn *in vitro*. Pharmacological studies *in vivo* show spinally applied nociceptin inhibits nociceptive neuronal transmission in the dorsal horn (Stanfa *et al*., 1996). In contrast, intracerebroventricular nociceptin produces hyperalgesia and can also reduce morphine analgesia (Reinscheid *et al*., 1995; Meunier, 1997 and references therein).

To elucidate the role of endogenous nociceptin, a selective ORL-1 antagonist is vital. [Phe^1 ψ(CH_2-NH)Gly^2]nociceptin-(1-13)-NH_2, a pseudopeptide analogue of nociceptin, is a competitive antagonist in guinea-pig ileum and mouse vas deferens (Guerrini *et al*., 1998). However, in Chinese hamster ovary (CHO) cells expressing the human ORL-1 receptor, [Phe^1 ψ(CH_2-NH)Gly^2]nociceptin-(1-13)-NH_2 is an agonist (Butor *et al*., 1998). Here we investigate the effects of intrathecal [Phe^1 ψ(CH_2-NH)Gly^2]nociceptin-(1-13)-NH_2 on spinal neurones in the anaesthetized rat, an *in vivo* model where nociceptin is inhibitory.

Methods Male Sprague-Dawley rats (200–250 g) were anaesthetized with halothane and maintained at 1.8% (see Dickenson *et al*., 1987 for full methods) and extracellular recordings made from single dorsal horn neurones responding to noxious and innocuous stimuli in L4–L5 segments. Responses were elicited from ipsilateral hindpaw receptive fields by transcutaneous electrical stimulation, at three times the C-fibre threshold. At 10-min intervals, a train of 16 stimuli (0.5 Hz) was given and post-stimulus histograms constructed. These evoked responses were separated by latency into Aβ-fibre evoked activity (0–20 ms), Aδ-fibre (20–90 ms), C-fibre (90–300 ms) and post-discharge (300–800 ms) and quantified. Responses evoked by the first stimulus of the train are referred to as 'input' and consist of the number of action potentials (90–300 ms) evoked by this stimulus. Wind-up, a measure of the enhanced neuronal response elicited by repetitive stimulation, was quantified as the difference between the total number of action potentials produced by the 16 stimuli (90–800 ms), and the input $\times 16$.

After control responses stabilized, cumulative doses of [Phe^1 ψ(CH_2-NH)Gly^2]nociceptin-(1-13)-NH_2 (1 μg, 25 μg, 75 μg and 125 μg) in 50 μl saline, were applied to the spinal cord and neuronal responses followed for 40 min. After maximum inhibition was reached, either 5 μg ($n=3$) or 10 μg ($n=4$) naloxone was applied intrathecally, followed by the highest dose of naloxone (50 μg). Each dose was followed for 40 min.

Statistical analysis of drug effects used one-way analysis of variance (ANOVA) and Fisher's protected least significant difference test. $P<0.05$ was considered significant.

Results The effect of [Phe^1 ψ(CH_2-NH)Gly^2]nociceptin-(1-13)-NH_2 was studied on 14 dorsal horn neurones (665 ± 42 μm depth from the cord surface). C- and Aδ-fibre evoked responses, input, wind-up and post discharge were preferentially inhibited by [Phe^1 ψ(CH_2-NH)Gly^2]nociceptin-(1-13)-NH_2, in a dose-related manner, whereas the Aβ-fibre evoked responses were not altered, suggesting selective actions on noxious evoked activity.

[Phe^1 ψ(CH_2-NH)Gly^2]nociceptin-(1-13)-NH_2 significantly inhibited the C-fibre evoked response ($P=0.009$, control = 266 ± 19 action potentials, Figure 1A). Inhibition by 25 μg of the drug was significant with the maximum effect seen with 75 μg of drug ($n=5$, $P<0.05$ for both). In contrast, the Aβ-fibre evoked response (control $=97\pm8$ action potentials) was not significantly affected by intrathecal [Phe^1 ψ(CH_2-NH)Gly^2]nociceptin-(1-13)-NH_2 (Figure 1A).

The input (control = 13 ± 8 action potentials) and wind-up (control = 190 ± 25) responses were consistently reduced by [Phe^1 ψ(CH_2-NH)Gly^2]nociceptin-(1-13)-NH_2, Figure 1B) but these effects did not reach significance. Post discharge, another measure of wind-up, was significantly reduced by [Phe^1 ψ(CH_2-NH)Gly^2]nociceptin-(1-13)-NH_2 ($P<0.05$, control = 124 ± 25 action potentials) after an initial facilitation with the lowest dose (Figure 1B). There was a trend towards inhibition of Aδ-fibre evoked responses (control = 42 ± 7 action potentials).

In the example shown in Figure 2, 5 μg naloxone partly reversed the inhibitory effect of 75 μg intrathecal [Phe^1 ψ(CH_2-NH)Gly^2]nociceptin-(1-13)-NH_2 on wind-up. Seven of the 14

[2] Author for correspondence.

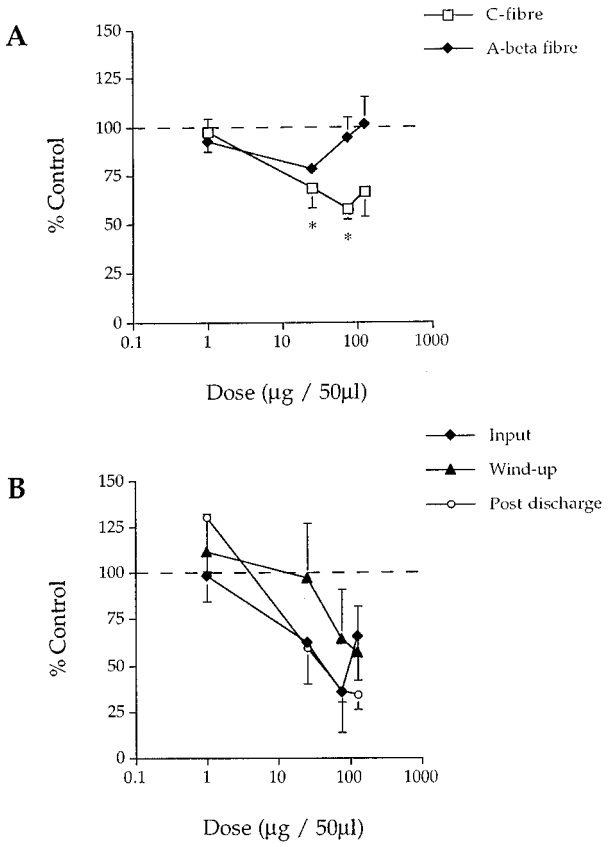

Figure 1 The effect of intrathecal [Phe^1 ψ(CH$_2$-NH)Gly^2]nociceptin-(1-13)-NH$_2$) on (A) C-fibre and Aβ-fibre evoked responses and (B) input, wind-up and post discharge. Data are shown as mean percentage control and vertical lines show s.e.mean. *$P<0.05$.

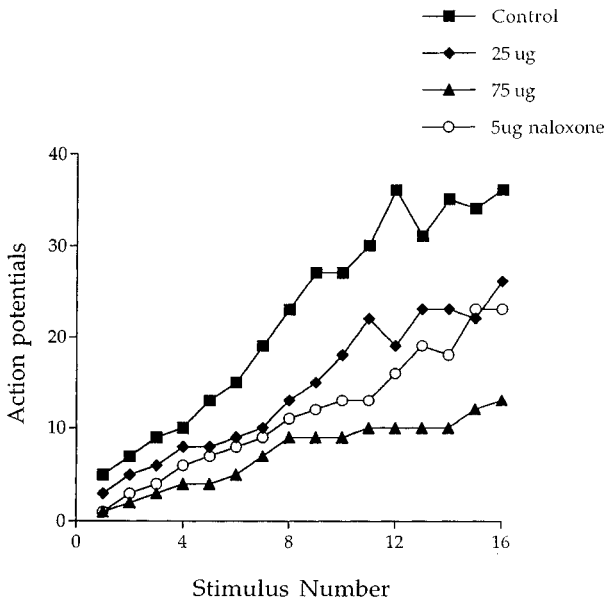

Figure 2 The effect of intrathecal [Phe^1 ψ(CH$_2$-NH)Gly^2]nociceptin-(1-13)-NH$_2$) (25 μg and 75 μg) on an individual neurone exhibiting wind-up. 5 μg naloxone, applied intrathecally, partially reversed the effect.

neurones studied were tested with a range of doses of naloxone (5 μg, 10 μg and 50 μg), which partially reversed the inhibitory effects of [Phe^1 ψ(CH$_2$-NH)Gly^2]nociceptin-(1-13)-NH$_2$. The highest dose of naloxone produced the greatest reversal. C-fibre evoked responses were reversed, on average, to 84$\pm$6% control; post discharge to 94$\pm$21%; wind-up to 108$\pm$22% and input to 68$\pm$10% of pre-drug control values.

Discussion This is the first study of [Phe^1 ψ(CH$_2$-NH)Gly^2]-nociceptin-(1-13)-NH$_2$, a putative antagonist at the ORL-1 receptor *in vivo*. Overall, [Phe^1 ψ(CH$_2$-NH)Gly^2]nociceptin-(1-13)-NH$_2$ had inhibitory actions, significantly inhibiting C-fibre evoked responses and post discharge but not Aβ-fibre evoked responses. This selectivity of action mirrored that of spinal nociceptin; a comparison of Figure 1 with Figure 1 in Stanfa *et al.* (1996) shows the similarity between the potency of the two drugs.

In contrast to nociceptin, [Phe^1 ψ(CH$_2$-NH)Gly^2]nociceptin-(1-13)-NH$_2$ tended to reduce the C-fibre input (related to the baseline C-fibre evoked response recorded for nociceptin), an effect seen with intrathecal opioids (Dickenson *et al.*, 1987). The inhibitory effects of [Phe^1 ψ(CH$_2$-NH)Gly^2]nociceptin-(1-13)-NH$_2$ were more susceptible to naloxone reversal than those of nociceptin. [Phe^1 ψ(CH$_2$-NH)Gly^2]nociceptin-(1-13)-NH$_2$ may therefore have some activity at spinal μ- or δ-opioid receptors although it has been thought to be selective for the peripheral ORL-1 receptor (Guerrini *et al.*, 1998).

In two peripheral preparations – the guinea-pig ileum and the mouse vas deferens – [Phe^1 ψ(CH$_2$-NH)Gly^2]nociceptin-(1-13)-NH$_2$ is an antagonist, blocking the effects of nociceptin. We have shown, in the rat spinal cord, that this pseudopeptide mimics nociceptin, and so is likely to be an agonist at the rat spinal ORL-1 receptor. Butor *et al.* (1998) have also shown agonist actions of this pseudopeptide on recombinant human ORL-1 receptors. These tissue dependent effects suggest that the cloned human receptor and that in rat spinal cord could differ from the receptors found in the guinea-pig ileum and mouse vas deferens. In support of this premise of receptor diversity, Mathis *et al.* (1997) have biochemical evidence for heterogeneity of the ORL-1 receptor in mouse brain.

K.C. is a postgraduate student and this work is funded by European Community Biomed award No. CT97 2317.

References

ANTON, B., FEIN, J., TO, T., LI, X., SILBERSTEIN, L. & EVANS, C.J. (1996). Immunohistochemical localization of ORL-1 in the central nervous system of the rat. *J. Comp. Neurol.*, **368,** 229–251.

BUTOR, J.-L., MOISAND, C., MOLLEREAU, C. & MEUNIER, J.-C. (1998). [Phe^1 ψ(CH$_2$-NH)Gly^2]nociceptin-(1-13)-NH$_2$ is an agonist of the nociceptin (ORL-1) receptor. *Eur. J. Pharmacol.*, In press.

DICKENSON, A.H., SULLIVAN, A., KNOX, R., ROQUES, B.P. & ZAJAC, J. (1987). Opioid receptor subtypes in the rat spinal cord: electrophysiological evidence for a role of mu and delta opioid receptor antagonists in the control of nociception. *Brain Res.*, **413,** 44–46.

GUERRINI, R., CALO, G., RIZZI, A., BIGONI, R., BIANCHI, C., SALVADORI, S. & REGOLI, D. (1998). A new selective antagonist of the nociceptin receptor. *Br. J. Pharmacol.*, **123,** 163–165.

MATHIS, J.P., RYAN-MORO, J., CHANG, A., HOM, J.S., SCHEINBERG, D.A. & PASTERNAK, G.W. (1997). Biochemical evidence for orphanin FQ/nociceptin receptor heterogeneity in mouse brain. *Biochem. Biophys. Res. Commun.,* **230,** 462–465.

MEUNIER, J.-C. (1997). Nociceptin/orphanin FQ and the opioid receptor-like ORL1 receptor. *Eur. J. Pharmacol.,* **340,** 1–15.

PELUSO, J., LAFORGE, K.S., MATTHES, H.W., KREEK, M.J., KIEFFER, B.L. & GAVÉRIAUX-RUFF, C. (1998). Distribution of nociceptin/orphanin FQ receptor transcript in human central nervous system and immune cells. *J. Neuroimmunol.,* **81,** 184–192.

REINSCHEID, R.K., NOTHAKER, H.-P., BOURSON, A., ARDATI, A., HENNINGSEN, R.A., BUNZOW, J.R., GRANDY, D.K., LANGEN, H., MONSMA JR, F.R. & CIVELLI, O. (1995). Orphanin FQ: A neuropeptide that activates an opioid-like G protein-coupled receptor. *Science,* **270,** 792–794.

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.

WICK, M.J., MINNERATH, S.R., LIN, X., ELDE, R., LAW, P.-Y. & LOH, H.H. (1994). Isolation of a novel cDNA encoding a putative membrane receptor with high homology to the cloned μ, δ and κ opioid receptors. *Mol. Brain Res.,* **27,** 37–44.

WILLIAMS, C.A., WU, S.Y., COOK, J. & DUN, N.J. (1998). Release of nociceptin-like substance from the rat spinal cord dorsal horn. *Neurosci. Lett.,* **244,** 141–144.

(Received June 9, 1998
Revised July 31, 1998
Accepted August 8, 1998)