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SPECIAL REPORT

# Inhibitory action of nociceptin on spinal dorsal horn neurones of the rat, *in vivo*

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Intrathecally administered nociceptin (5, 50, 225 μg) dose-relatedly inhibited the C-fibre evoked wind-up and post-discharge of dorsal horn neurones, but not the baseline C-fibre evoked responses. Spinal naloxone 50 μg, but not 10 μg, reversed the effects of nociceptin. Thus the antinociceptive role of nociceptin in the spinal cord differs from that of classical opioids.

**Keywords:** Nociceptin; antinociception; spinal cord; wind-up; C-fibres; naloxone

**Introduction** The cloning of the μ, δ and κ opioid receptors led to the discovery of a new opioid receptor, the orphan or the opioid receptor like-1 ($ORL_1$) receptor (see references in Meunier *et al.*, 1995; Reinscheid *et al.*, 1995). The heptadecapeptide, orphanin FQ/nociceptin (hereafter nociceptin) is an endogenous ligand for the $ORL_1$ receptor (Meunier *et al.*, 1995; Reinscheid *et al.*, 1995). Behavioural studies have shown that intracerebroventricular nociceptin, unlike other opioids, is pro-nociceptive (Meunier *et al.*, 1995; Reinscheid *et al.*, 1995). Since the transcript encoding the $ORL_1$ receptor is present in the spinal cord (Wick *et al.*, 1994), we have investigated the effect of spinal administration of nociceptin on sensory processing in the dorsal horn.

**Methods** Sprague-Dawley rats (~225 g) were anaesthetized and then maintained on 1.7% halothane. Following a laminectomay, extracellular recordings were made from dorsal horn neurones responding to Aβ- and C-fibre cutaneous afferents from the hindpaw following transcutaneous electrical stimulation (see Chapman *et al.*, 1994). Responses were elicited by a train of 16 stimuli (3 times C-fibre threshold, 0.5 Hz), at 5 min intervals, and post-stimulus histograms constructed. The repetitive stimuli caused wind-up, an enhanced neuronal response (see Figure 2b) which leads to a post-discharge. The evoked responses were separated and quantified on the basis of threshold and latency: Aβ-fibre evoked activity from 0-20 ms; C-fibres (90-300 ms) and post-discharges (300-800 ms). The baseline response evoked by C-fibre stimulation was calculated as the number of action potentials produced by the first stimulus ×16. Wind-up was the difference between the total number of action potentials (90 ms to 800 ms) produced by the sixteen stimuli and the baseline.

After stable control responses, cumulative doses of nociceptin (Tocris Cookson) (5, 50 and 225 μg (2.8, 27.8 and 124 nmol, respectively), in 50 μl of saline) were applied onto the lumbar spinal cord at 40 min intervals. The ability of naloxone (10 or 50 μg in 50 μl of saline) to reverse the effects of 225 μg of nociceptin was studied. Data are expressed as percentages of control response ± s.e.mean. Statistical analysis was by one way analysis of variance (ANOVA), and Fischer's PLSD (protected least significant differences) test for comparisons.

**Results** The effect of nociceptin was studied on 14 dorsal horn neurones (depth 618 ± 69 μm from spinal surface).

Intrathecal nociceptin (5, 50 and 225 μg) had limited effects on the afferent C-fibre evoked responses (control = 314 ± 39 action potentials) of the neurones (Figure 1a); only 225 μg of nociceptin appeared to reduce the C-fibre evoked response (62 ± 15% of control, $P = 0.06$, $n = 8$). Analysis of individual neurones revealed that nociceptin had non-uniform effects especially at higher concentrations. Two out of the eight neurones were consistently facilitated by 225 μg nociceptin (Figure 2a).

The baseline C-fibre response of the population of neurones was not significantly influenced by nociceptin (Figure 1b). Again, as for the afferent C-fibre evoked response, a non-uniform effect of nociceptin was apparent. Some facilitations were seen with lower doses, and the inhibition of the baseline by 225 μg of nociceptin ranged widely (2-81% ($n = 8$)).

In contrast to the effects of nociceptin on the acute C-fibre responses, wind-up (control = 244 ± 32 action potentials) was consistently reduced by nociceptin (Figure 1b). Nociceptin, 50 μg tended to inhibit (65 ± 20%, $P < 0.07$, $n = 14$) and 225 μg significantly reduced the wind-up of these neurones (48 ± 16% of control, $P < 0.01$, $n = 8$, Figures 1b and 2b). In keeping with the effect on wind-up, the post-discharge (control = 176 ± 24 action potentials) was also significantly reduced by the highest dose of nociceptin (41 ± 12% of control, $P < 0.04$, $n = 8$, Figure 1b).

Overall, nociceptin did not significantly ($P = 0.07$) influence the Aβ-fibre evoked responses of the dorsal horn neurones (control = 86 ± 9 action potentials) although there was a trend towards a reduction with 225 μg of nociceptin (67 ± 9% of control, $n = 8$, Figure 1a).

Naloxone (10 μg) did not reverse the inhibitory actions of 225 μg nociceptin on any responses ($n = 4$). However, an extremely high dose of intrathecal naloxone (50 μg) fully reversed the depressive effects ($n = 2$).

**Discussion** This study is the first to demonstrate that nociceptin selectively modulates spinal nociceptive events by preferentially reducing wind-up and post-discharge of the neurones. This contrasts with reports of hyperalgesia following supraspinal administration (Meunier *et al.*, 1995; Reinscheid *et al.*, 1995) and no effect after spinal application (Reinscheid *et al.*, 1995). Our results are in keeping with those from *in vitro* studies demonstrating inhibitory effects of nociceptin on dorsal raphe nucleus neurones (Vaughan & Christie, 1996) and neuroblastoma cells (Connor *et al.*, 1996).

The predominant effect of nociceptin on wind-up and post-discharge of dorsal horn neurones differs from the clear inhibition of the baseline C-fibre response produced by morphine and endogenous enkephalins, acting on μ- and δ-opioid receptors (Dickenson, 1991; Chapman *et al.*, 1994). The bidirectional effects of nociceptin on the afferent C-fibre evoked response are reminiscent of intrathecal dynorphin $A_{1-13}$ (Dickenson, 1991), the only other endogenous opioid that has any affinity at the $ORL_1$ receptor (Zhang & Yu, 1995).

Although the potency of nociceptin was similar to other

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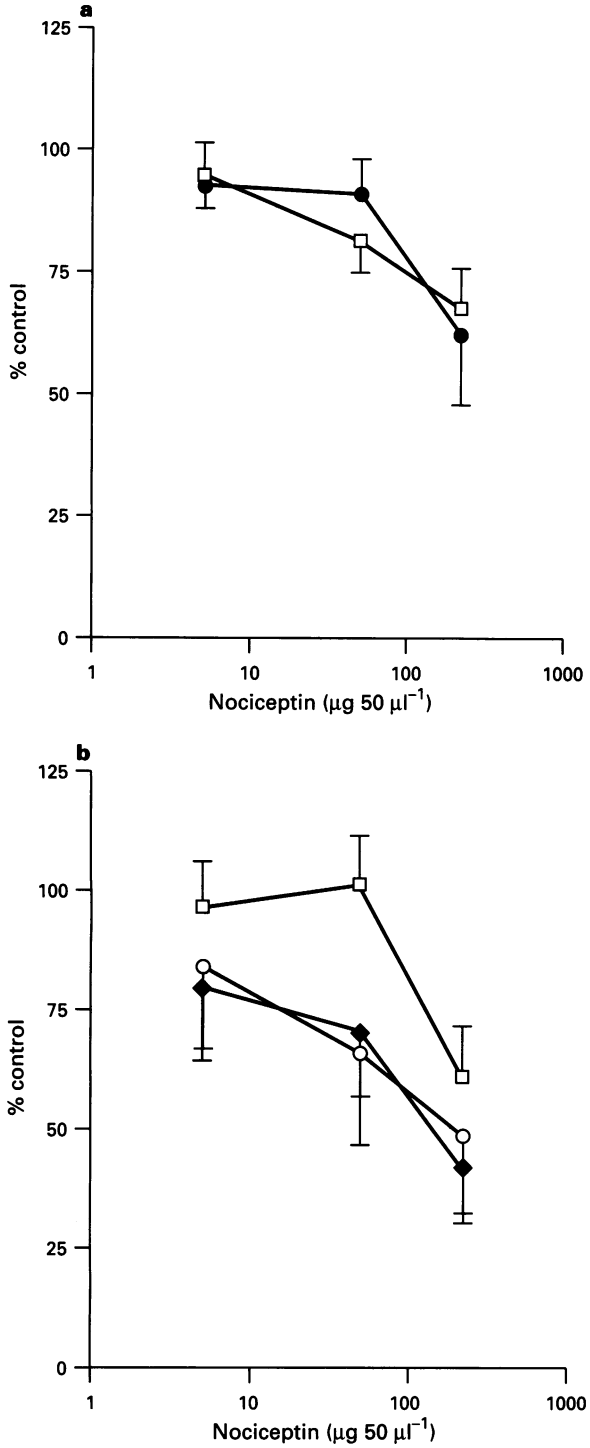


**Figure 1** The effect of intrathecal nociceptin on the (a) C-fibre (●) and Aβ-fibre (□) and (b) baseline C-fibre evoked response (□), wind-up (○) and post-discharge (◆) ($n=8-14$ neurones per dose). Note the preferential inhibition of the wind-up and post-discharge of the neurones. Data shown are means and vertical lines indicate s.e.mean.

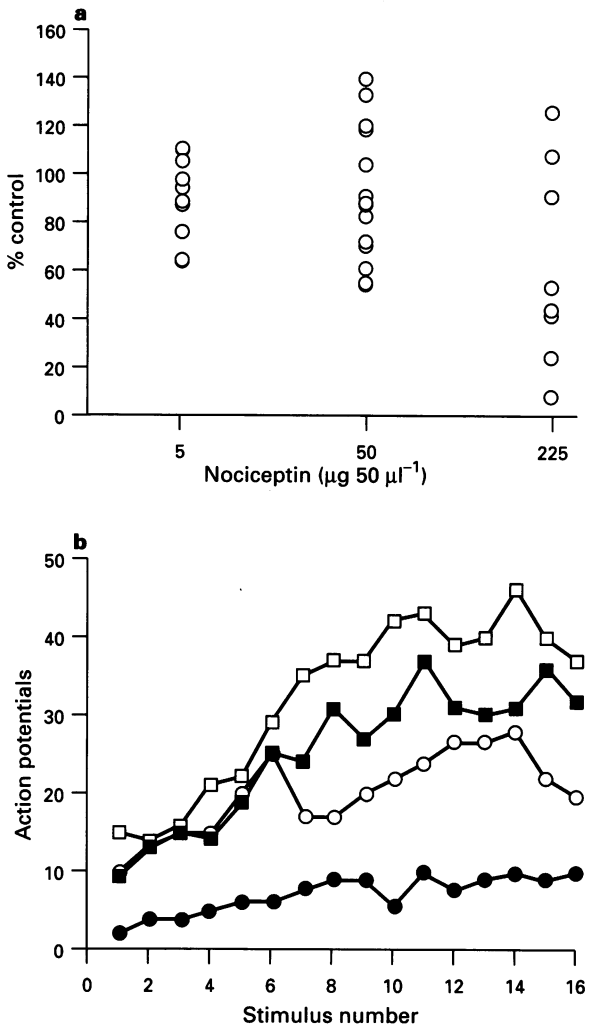


**Figure 2** (a) The non-uniform effect of nociceptin on the C-fibre evoked responses of individual neurones expressed as percentages of individual pre-drug controls: some neurones were facilitated following nociceptin (225 μg). (b) The maximal effect (30–40 min post-administration) of nociceptin (50 μg (○), 225 μg (●)) on the response of an individual neurone displaying wind-up (■). Nociceptin (50 μg) reduced the wind-up, but not the baseline C-fibre response. Intrathecal administration of naloxone (50 μg, □) reversed the effect of nociceptin (225 μg).

opioid peptides, naloxone sensitivity was much lower (Dickenson, 1991), in keeping with the low potency of naloxone at the $ORL_1$ receptor (Zhang & Yu, 1995). The effects of nociceptin on the various components of the overall C-fibre evoked responses of the neurones implies different locations or physiological functions of the $ORL_1$ receptor and $\mu/\delta$ opioid receptors. We obtained little evidence that nociceptin is anything other than a predominantly inhibitory peptide at the spinal level.

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