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SPECIAL REPORT

Distinct inhibitory effects of spinal endomorphin-1 and endomorphin-2 on evoked dorsal horn neuronal responses in the rat

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Intrathecal endomorphin-1 and endomorphin-2 (0.25–50 μg) dose-relatedly reduced all components of electrical evoked C-fibre responses of spinal neurones. These effects were partially reversed by naloxone. Endomorphin-1, but not endomorphin-2, dose-relatedly reduced the Aβ-fibre evoked responses. Peak inhibitory effects of endomorphin-1 and -2 were at 15–20 min post-administration. Thus spinal endomorphin-2 had selective effects on noxious responses, whereas endomorphin-1 was non-selective.

Keywords: Antinociception; endomorphin-1; endomorphin-2; intrathecal; spinal neurones

Introduction The recently discovered peptides (endomorphin-1 and endomorphin-2) with high affinity and selectivity for μ-opioid receptors may be the endogenous μ-opioid receptor ligands (Zadina *et al*., 1997). In *in vitro* studies endomorphin-1 was a more potent μ-opioid agonist than DAMGO and endomorphin-1 was as potent as morphine in producing naloxone-reversible analgesia (Zadina *et al*., 1997).

The presence of endogenous endomorphin-1 or endomorphin-2 at the level of the spinal cord has yet to be demonstrated, but these peptides may be the sought after endogenous ligands for spinal μ-opioid receptors.

We have compared the effect of exogenous spinal endomorphin-1 and endomorphin-2 on electrically evoked noxious and innocuous responses of dorsal horn neurones of the rat.

Methods Techniques used have been described previously (Chapman *et al*., 1994). Sprague-Dawley rats (200–250 g, $n=18$) were anaesthetized (halothane in 66% N_2O/33% O_2) and extracellular recordings of dorsal horn neurones were made. Neurones responded to Aβ- and C-fibre afferent inputs from the hindpaw following a train of 16 electrical stimuli at 0.5 Hz (3× threshold current for C-fibre and for Aβ-fibre evoked activity) and post-stimulus histograms were constructed. Evoked responses were separated and quantified by thresholds and latencies: Aβ-fibre: 0–20 ms post stimulus; C-fibre: 90–300 ms and post-discharges at 300–800 ms. Non-potentiated responses of the dorsal horn neurones evoked by C-fibre stimulation were calculated as the number of action potentials produced by the first stimulus multiplied by the total number of stimuli (sixteen).

Following control responses (less than 10% variance) endomorphin-1 (0.25–50 μg [0.4–82 nmol], $n=9$) and endomorphin-2 (0.25–50 μg [0.4–87 nmol], $n=9$) in 50 μl of saline, both from Tocris Cookson, were applied cumulatively onto the spinal cord in a total of 18 rats. Drug effects were quantified at 2, 5, 10, 20, 30 and 40 min post-drug administration and naloxone (1 μg in 50 μl of saline) was given at this latter time to test reversibility of the effects of 50 μg of endomorphin-1 and endomorphin-2. Data are presented as percentage inhibition of the control response ± s.e.mean. The effects of the highest concentration of endomorphin-1 and endomorphin-2 were compared to the matched control values with a paired Student's *t* test.

Results The mean depth of the two groups of spinal neurones ($n=9$, for each) studied with endomorphin-1 and endomorphin-2 were 830 ± 55 μm and 822 ± 54 μm, respectively; mean thresholds were 0.1 ± 0.03 mA and 0.1 ± 0.02 mA (Aβ-fibre evoked responses) and 1.1 ± 0.2 mA and 1 ± 0.2mA (C-fibres), respectively.

The evoked C-fibre responses of the two groups of neurones were similarly reduced by intrathecal endomorphin-1 and -2 (Figure 1a). The effects of 50 μg endomorphin-1 and -2 on the C-fibre evoked neuronal responses were significant ($P<0.05$, for both) and partially reversed by intrathecal naloxone (1 μg). Peak effects of endomorphin-1 and -2 (8, 16 and 50 μg) on C-fibre evoked responses of spinal neurones were at 22 ± 4, 25 ± 5 and 25 ± 3 min after endomorphin-1 administration, respectively, and at 19 ± 5, 16 ± 5 and 25 ± 6 min after endomorphin-2 administration, respectively.

The non-potentiated and post-discharge components of the C-fibre evoked responses of both groups of neurones were significantly reduced by the highest concentrations of endomorphin-1 and -2 studied (Figure 2a and b, respectively) ($P<0.05$, in all cases) and partially reversed by intrathecal naloxone. Spinal administration of low doses of endomorphin-1, but not endomorphin-2, facilitated the non-potentiated, but not the post-discharge, component of the C-fibre evoked neuronal response.

Endomorphin-1, but not endomorphin-2, reduced the electrical Aβ-fibre evoked responses of spinal neurones, which were partially reversed by intrathecal naloxone (1 μg) (Figure 1b). Peak effects of 8, 16 and 50 μg of endomorphin-1 on the Aβ-fibre evoked neuronal responses were at 26 ± 5, 16 ± 3 and 23 ± 6 min, respectively.

Discussion Spinally administered endomorphin-1 and endomorphin-2 reduced C-fibre evoked neuronal responses. Only endomorphin-1 influenced Aβ-fibre evoked neuronal responses. Endomorphin-1, but not endomorphin-2, produced classical low dose μ-opioid agonist facilitations of the non-potentiated component of the C-fibre evoked response (Dickenson *et al*., 1987). Higher concentrations of endomorphin-1 and endomorphin-2 had similar inhibitory effects on the non-potentiated component of the C-fibre evoked neuronal responses, akin to other μ-opioids (Dickenson *et al*., 1987). The effects of spinal endomorphin-1 on the non-potentiated C-fibre evoked neuronal response are comparable to the behavioural analgesic effects of intrathecal endomorphin-1 in mice (Zadina *et al*., 1997). Higher concentrations of endomorphin-1 had greater effects on the non-potentiated, as compared to the post-discharge, com-

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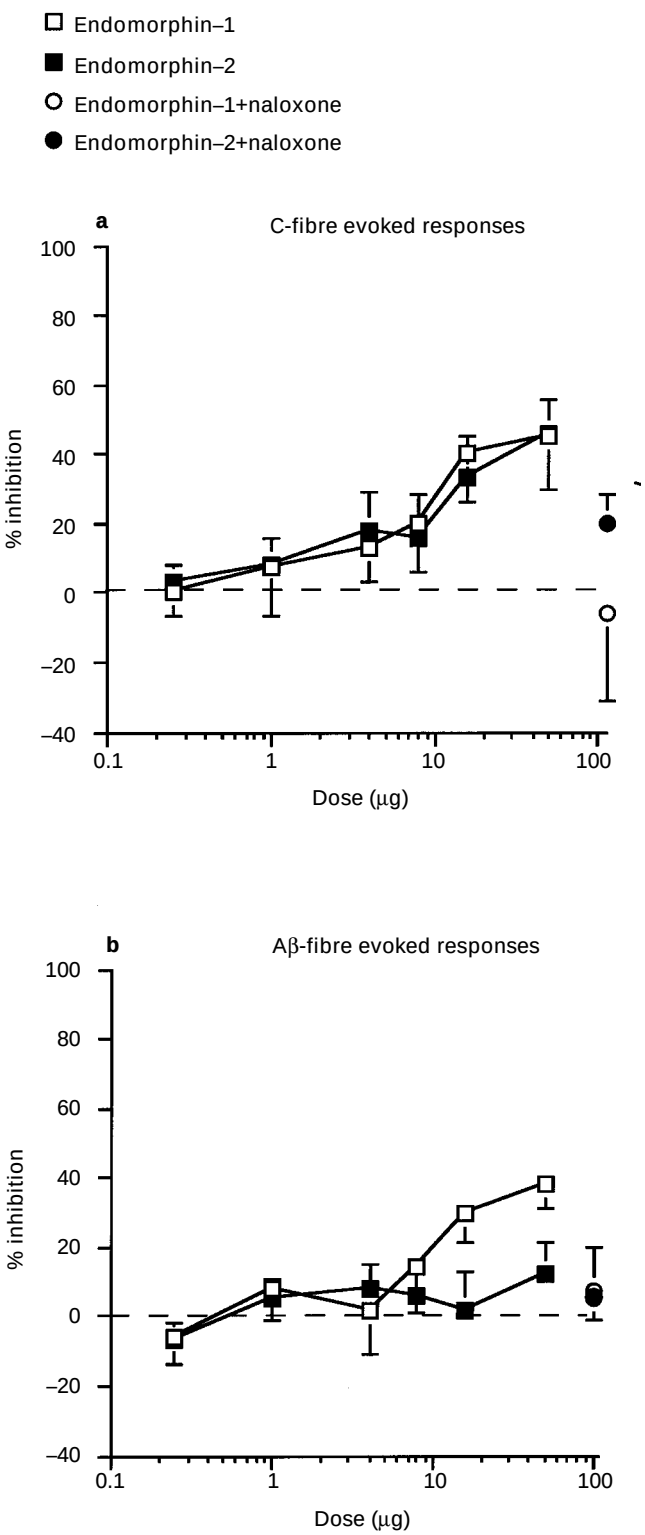

Figure 1 (a) Fifty micrograms of endomorphin-1 ($n=9$) and endomorphin-2 ($n=9$) significantly reduced the C-fibre evoked neuronal responses ($P<0.05$ as compared to control, for both). These effects of endomorphin-1 and endomorphin-2 were completely reversed by intrathecal naloxone (1 μg). Mean control C-fibre evoked neuronal responses for endomorphin-1 and endomorphin-2 were 374 ± 67 and 370 ± 32 action potentials, respectively. (b) Higher concentrations of endomorphin-1 ($n=9$), but not endomorphin-2 ($n=9$), reduced Aβ-fibre evoked neuronal responses in a naloxone reversible manner. Mean control Aβ-fibre evoked neuronal responses for endomorphin-1 and endomorphin-2 were 72 ± 9 and 115 ± 9 action potentials, respectively. In (a) and (b) vertical lines show s.e.mean.

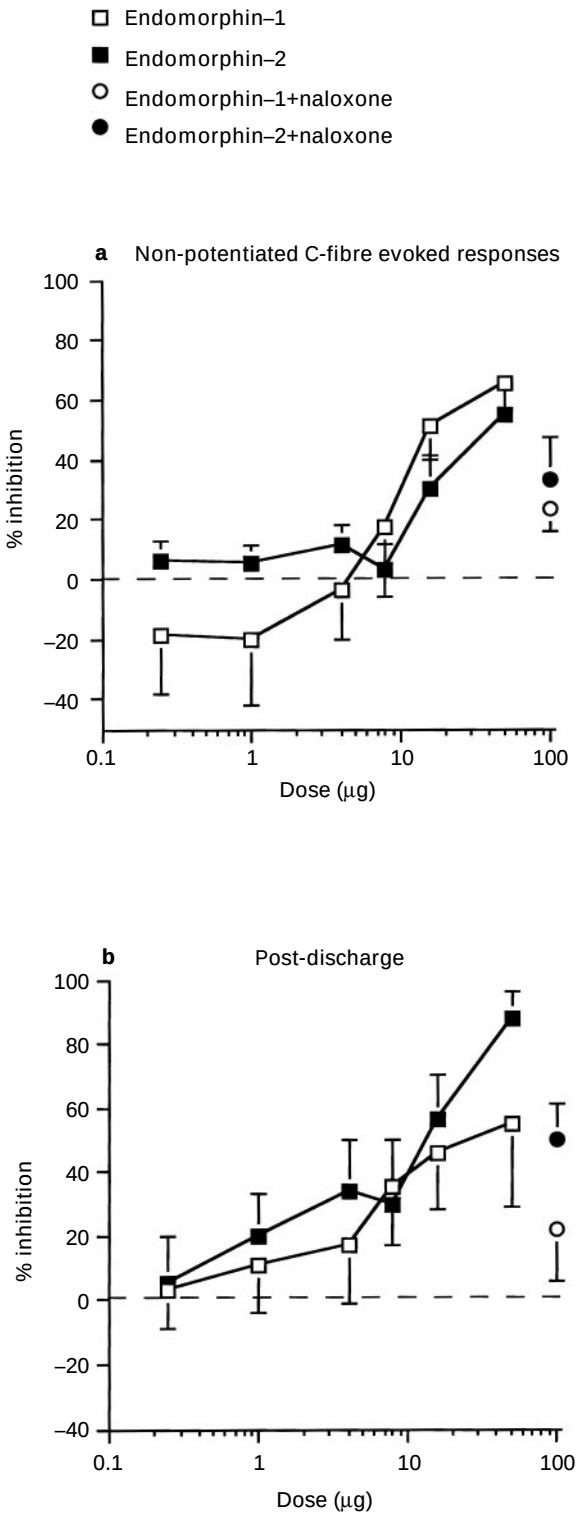

Figure 2 (a) The highest concentration of endomorphin-1 ($n=9$) and endomorphin-2 ($n=9$) significantly reduced the non-potentiated C-fibre evoked neuronal responses ($P<0.05$, for both); these effects of endomorphin-1 and endomorphin-2 were partially reversed by intrathecal naloxone (1 μg). Mean control evoked non-potentiated neuronal responses for endomorphin-1 and endomorphin-2 were 448 ± 95 and 303 ± 47 action potentials, respectively. (b) The highest concentration of endomorphin-1 ($n=9$) and endomorphin-2 ($n=9$) significantly reduced the post-discharge responses ($P<0.05$, for both); these effects were partially reversed by intrathecal naloxone (1 μg). Mean control evoked post-discharge neuronal responses for endomorphin-1 and endomorphin-2 were 313 ± 53 and 213 ± 31 action potentials, respectively. In (a) and (b) vertical lines show s.e.mean.

ponent of the C-fibre evoked neuronal response, this was the converse for endomorphin-2.

The inhibitory effects of endomorphin-1 and endomorphin-2 were partially reversed by naloxone. The timing of the peak effects of the peptides are similar to the behavioural effects (Zadina *et al*., 1997).

Endomorphin-1 and endomorphin-2 had differential effects on noxious versus innocuous responses, and on the two components of the C-fibre evoked neuronal responses. These differences are hard to explain but may arise from the relative selectivity of the peptides for opioid receptors (Zadina *et al*., 1997), possible multiple μ-opioid receptor subtypes, or access to the site(s) of action. Overall, the two putative endogenous μ-opioid agonists had clearly discernible physiological actions.

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