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# Involvement of glutamate NMDA and AMPA receptors, glial cells and IL-1β in the spinal hyperalgesia evoked by the chemokine CCL2 in mice

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ABSTRACT

We study here the involvement of excitatory amino acid receptors, glial cell activation and IL-1β release in the spinal hyperalgesia evoked by the chemokine CCL2 (MCP-1). Three hours after the intrathecal administration of CCL2 (1–100 ng), mice exhibit dose-dependent thermal hyperalgesia, that was inhibited by the coadministration of the antagonist of chemokine receptor type 2 (CCR2) RS504393 (0.3–3 μg). To assess the involvement of excitatory amino acid receptor sensitisation, CCL2 was coadministered with CPP (0.3–3 ng) and NBQX (25–250 ng), antagonists of NMDA and AMPA receptors, respectively. Both drugs blocked CCL2-evoked hyperalgesia, strongly suggesting that CCL2 evokes in vivo NMDA and AMPA receptor sensitisation, as previously described in electrophysiological studies. Furthermore, this rapid induction of CCL2-mediated hyperalgesia was blocked by the previous acute administration of the microglial inhibitor minocyclin (4.9 μg) or the astroglial inhibitor L-aminoadipate (1.6 μg). Since IL-1β can be released by activated glial cells we tested whether this cytokine could be underlying the spinal sensitisation induced by CCL2. The administration of the type I IL-1 receptor antagonist, IL-1ra (3–30 μg), partially prevented CCL2-evoked hyperalgesia. Finally, to elucidate if IL-1β could produce NMDA and AMPA receptor sensitisation by itself, we performed experiments in which this cytokine was i.t. administered. Thermal hyperalgesia induced by IL-1β (30 pg) was completely prevented by the coadministration of CPP (3 ng) but unaffected by NBQX (250 ng). Globally, our results suggest that spinal CCL2 induces thermal hyperalgesia by sensitising NMDA and AMPA receptors in a process that involves glial activation and IL-1β release.



Chemokines are a wide family of endogenous peptides whose involvement in macrophage/monocyte chemotaxis is well established. Besides this role, their direct involvement in nociceptive processes is being increasingly understood [1,5,16]. The activation of chemokine receptor type 2 (CCR2) has been involved in painful reactions associated to different pathological states, especially after nerve injury [5]. Studies focussed on CCR2 expression along the nociceptive system have demonstrated its presence in DRG [8] and spinal cord both in neurons [6], microglia [2] and astroglia [11].

CCL2, also known as monocyte chemoattractant protein 1 (MCP-1), is the endogenous ligand that preferentially binds CCR2 [8,13] and it is also expressed in primary sensory neurons [4,23] as well as in the spinal cord [4,6,24]. The role of CCL2 in pain modulation during pathological states is supported by its augmented expression after neuropathic [6,7,24], inflammatory [25] or neoplastic [10] processes and by the analgesic properties exhibited by CCR2 antagonists [21]. In accordance, the intrathecal administration of CCL2 produces spinal sensitisation and behavioural hyperalgesic reactions both in mice and rats [4,6,23,24].

Several mechanisms are probably involved in CCL2-evoked spinal hyperalgesia. Previous experiments based on patch-clamp recordings performed in spinal neurons have demonstrated that AMPA and NMDA receptor currents are enhanced in response to CCL2 [6]. Furthermore, apart from its neural action, both astroglia and microglia, two types of glial cells able to evoke spinal sensitisation, can be activated in response to CCR2 stimulation [17,24]. Aiming to explore if some of these facts described in vitro could account for in vivo hyperalgesia evoked by the spinal administration of CCL2 in mice, we have designed experiments to assess the involvement of AMPA or NMDA receptors, glial cell activation and IL-1β in this response.

Six to eight week old CD-1 Swiss male mice maintained in the Animalario of the Universidad de Oviedo (Reg. 33044 13A) were used. The experimental procedures were approved by the Comité Ético de Experimentación Animal de la Universidad de Oviedo (Asturias, Spain). CCL2 (MCP-1 from mouse, Sigma), (R)-CPP (Tocris), NBQX (Tocris), minocycline hydrochloride (Sigma), L-2-aminoadipic acid (L-AA, Sigma) and IL-1ra (R&D) were dissolved

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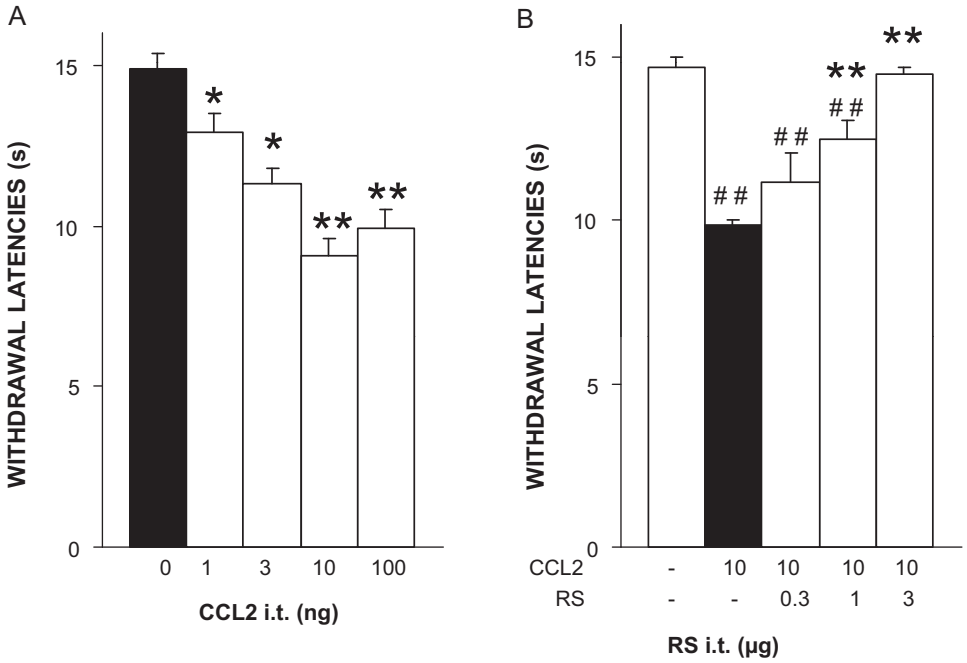


**Fig. 1.** (A) Decrease of withdrawal latencies measured by the unilateral hot plate test in mice after the i.t. administration of CCL2 (1–100 ng). (B) Inhibition of the hyperalgesic effect measured 3 h after the i.t. injection of 10 ng of CCL2 by the coadministration of the CCR2 antagonist RS504393 (0.3–3 μg). In both cases, mice that did not receive the administration of any drug were treated with i.t. vehicle. Each bar represents the mean ± SEM ($n$ = 5–6). *$P$ < 0.05, **$P$ < 0.01 compared with vehicle-treated group (A) or with CCL2-treated group (B), Dunnett's $t$ test. ##$P$ < 0.01 compared with vehicle-treated group, Newman–Keuls test.

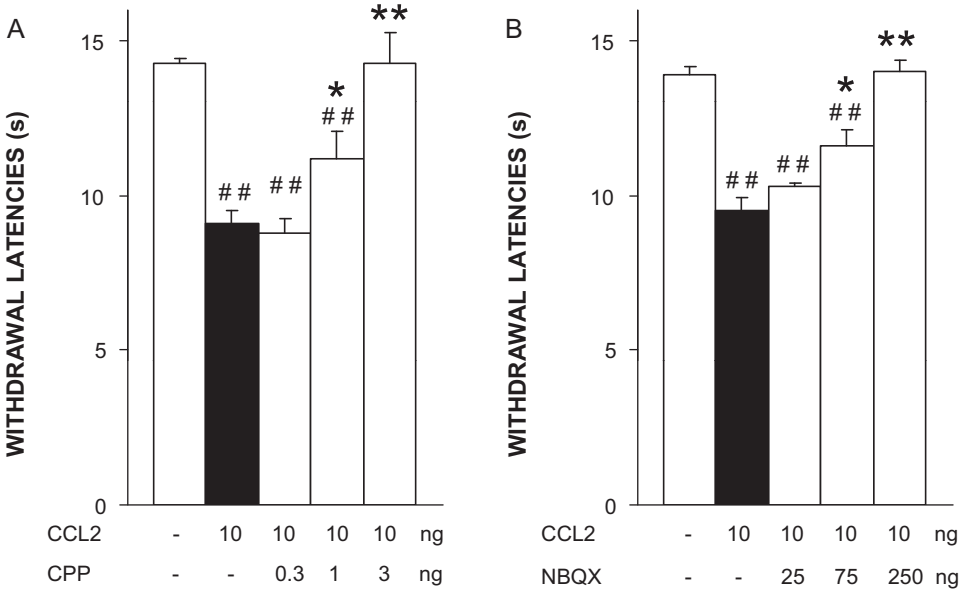


**Fig. 2.** Inhibition of the hyperalgesic effect evoked 3 h after the i.t. injection of 10 ng of CCL2 by the coadministration of the NMDA receptor antagonist CPP (0.3–3 ng) (A) or the AMPA receptor antagonist NBQX (25–250 ng) (B). In both cases, mice that did not receive the administration of any drug were treated with i.t. vehicle. Each bar represents the mean ± SEM ($n$ = 5–6). *$P$ < 0.05, **$P$ < 0.01 compared with CCL2-treated group, Dunnett's $t$ test; and ##$P$ < 0.01 compared with vehicle-treated group, Newman–Keuls test.

in saline and administered intrathecally (i.t.). The CCR2 antagonist RS504393 (Tocris) was dissolved in DMSO and diluted in saline to achieve a maximal DMSO concentration of 1%. For i.t. injections, a lumbar incision was made, and the tip of a 30-gauge needle connected to a Hamilton microsyringe was introduced at the level of L5–L6 to enable injections in a maximal volume of 5 μl [15].

Thermal withdrawal latencies were assessed by placing the plantar side of the tested paw on a hot plate surface (51 ± 0.5 °C) as previously described [15]. Briefly, withdrawal latencies were measured in each hind paw and the mean of the values obtained in both paws was calculated. The cut off was established in 30 s. Intergroup comparisons were made by an initial one-way analysis of variance followed by the Dunnett's $t$ test in the dose-effect curves or by the Newman–Keuls test for multiple groups. The level of significance was set at $P$ < 0.05.

The i.t. administration of CCL2 (1–100 ng) evokes a dose-dependent decrease of thermal withdrawal latencies. Although measurements were performed 3 h after CCL2 administration, we have checked in previous experiments that a single administration of this chemokine produces thermal hyperalgesic responses that