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Rapid communication

Formalin-induced pain is reduced in σ_1 receptor knockout mice

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Abstract

The role of σ_1 receptors in non-acute pain has not been explored. In this study we show that both phases of formalin-induced pain were reduced by approximately 55% in σ_1 receptor knockout mice in comparison to wild-type animals. These results suggest that the tonic pain induced by formalin is altered in mice lacking σ_1 receptors, and highlight the potential usefulness of further studies of the role of σ_1 receptors in models of non-acute pain.

Keywords: σ_1 Receptor; Formalin; Non-acute pain

Treatment with σ_1 receptor antisense oligodeoxynucleotides enhanced the antinociception produced by agonists of μ-, δ- and κ-opioid receptors in tail flick tests (Mei and Pasternak, 2002), which suggests that σ_1 receptors modulate opioid-induced antinociception in models of acute pain. However, the role of σ_1 receptors in non-acute pain has not been explored. Recently σ_1 receptor knockout mice became available (Langa et al., 2003), which offers new possibilities for testing the role of σ_1 receptors in nociception.

Activation of *N*-methyl-D-aspartate (NMDA) receptors is involved in several types of non-acute pain such as the tonic pain induced by formalin and different types of chronic pain (Coderre and Melzack, 1992; Petrenko et al., 2003). Because σ_1 receptor agonists potentiated NMDA-induced activation of neuronal firing (Debonnel and de Montigny, 1996), it can be hypothesized that NMDA-receptor-dependent types of pain may be reduced in σ_1 receptor knockout mice. To test this hypothesis we compared formalin-induced pain in σ_1 receptor knockout and wild-type mice.

Experiments were performed in 7- to 9-week-old female σ_1 receptor knockout and wild-type mice provided by Laboratorios Dr. Esteve, S.A., and obtained as described previously (Langa et al., 2003). To ensure homogeneity of the genetic background of the mice, the first generation of heterozygotes was bred for 5 generations on a C57BL/6J (Harlan Interfauna Iberica, S.A., Spain) background, with selection for the mutant σ_1 gene at each generation. Fifth generation heterozygotes were bred together to generate the mice used in this study. Animals were housed in rooms at 22±1 °C, with light from 08.00 to 20.00 h, and food and water were freely available. Testing took place from 09.00 to 15.00 h. All animal procedures complied with the guidelines of EEC Council Directive 86/609 and were approved by the University of Granada Ethics Committee.

A commercially available (Panreac, S.A., Spain) concentrated formalin solution was diluted with saline to the appropriate concentration (1.25%). Formalin test was performed as described previously (Rosland et al., 1990) with slight modifications. Briefly, 20 μl of the formalin solution was injected s.c. into the dorsal surface of the right hind paw of the mouse, using a Hamilton microsyringe with a $30^{1/2}$-gauge needle. The time spent in licking or biting the

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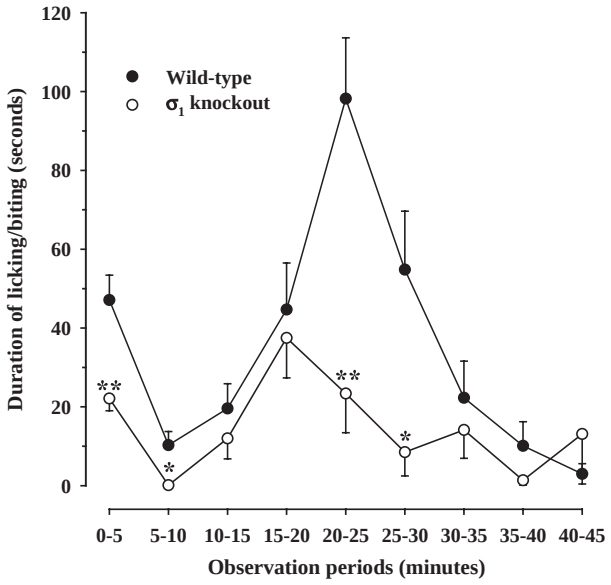

Fig. 1. Time-course of the pain response (duration of licking or biting of the treated paw) induced by the intraplantar injection of 1.25% formalin to σ_1 receptor knockout (○) and wild-type (●) mice. Each point represents the mean±S.E.M. of the data obtained in 8 animals. Statistically significant differences between the responses in the two types of mice: *P<0.05; **P<0.01 (Student's t test).

injected paw during 45 min (divided into 9 periods of 5 min each) after the injection was measured as an indicator of the pain response. Mean values for both types of animal were compared with Student's t test for unpaired samples, and the difference was considered statistically significant when P<0.05.

The intraplantar injection of formalin produced a biphasic pain response in wild-type animals with a first phase of acute pain during the first 10 min after the injection and a second phase which began after 10–15 min and lasted 45 min (Fig. 1). In σ_1 receptor knockout mice the response to formalin was markedly inhibited (Fig. 1). The duration of first phase pain was reduced from 57.1±6.3 s in wild-type animals to 22.13±3.11 s in σ_1 receptor knockout mice, and the duration of second phase pain was reduced from 242.7±27.57 s to 110.1±24.41 s. These results suggest that σ_1 receptors are necessary for the full expression of formalin-induced pain.

First phase pain induced by formalin is postulated to be due to the direct activation of nociceptors, whereas second phase pain appears to be dependent on the combination of an inflammatory reaction in the peripheral tissue and functional changes in the dorsal horn neurons of the spinal cord, which become sensitized to nociceptive stimuli (Rosland et al., 1990; Coderre and Melzack, 1992). Our results suggest that σ_1 receptors are involved not only in the acute (nociceptive) pain of the first phase, but also in the inflammatory pain, spinal cord neuronal sensitization, or both, which occur during the second phase. Because NMDA receptor activation plays an important role in formalin-induced pain (Coderre and Melzack, 1992; Chaplan et al., 1997), the results obtained apparently support our hypothesis, and can be explained (at least partially) if σ_1 receptors facilitate the expression of formalin-induced pain through their known ability to modulate NMDA-mediated responses (Debonnel and de Montigny, 1996). If this is the case, other types of pain involving NMDA receptors would be expected to be reduced in σ_1 receptor knockout animals or in wild-type animals treated with σ_1 receptor antagonists. We are now doing new experiments to test both hypotheses.

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