Selective Cannabinoid CB₁ Receptor Activation Inhibits Spinal Nociceptive Transmission In Vivo

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Kelly, Sara and Victoria Chapman. Selective cannabinoid CB₁ receptor activation inhibits spinal nociceptive transmission in vivo. J Neurophysiol 86: 3061–3064, 2001. Cannabinoids (CB₁ receptors) are located at CNS sites, including the spinal cord, involved in somatosensory processing. Analgesia is one of the tetrad of behaviors associated with cannabinoid agonists. Here, effects of a potent cannabinoid CB₁ receptor agonist arachidonyl-2-chloroethylamide (ACEA) on evoked responses of dorsal horn neurons in anesthetized rats were investigated. Extracellular recordings of convergent dorsal horn neurons were made in halothane anesthetized Sprague-Dawley rats (n = 16). Effects of spinal application of ACEA on electrically evoked responses of dorsal horn neurons were studied. Mean maximal effects of 0.5, 5, 50, and 500 ng/50 μl ACEA on the C-fiber-mediated postdischarge response were 79 ± 6, 62 ± 10, and 54 ± 7% (P < 0.01), 45 ± 6% (P < 0.01), of control, respectively. ACEA (500 ng/50 μl) also reduced the C-fiber-evoked nonpotentiated responses of neurons (59 ± 9% of control, P < 0.05) and Aδ-fiber-evoked responses of neurons (68 ± 10% of control, P < 0.01). Minor effects of ACEA on Aβ-fiber-evoked responses were observed. Spinal preadministration of the selective CB₁ receptor antagonist SR141716A (0.01 μg/50 μl) significantly reduced effects of ACEA (500 ng/50 μl) on postdischarge responses of dorsal horn neurons. This study demonstrates that spinal CB₁ receptors modulate the transmission of C- and Aδ-fiber-evoked responses in anesthetized rats; this may reflect pre- and/or postsynaptic effects of cannabinoids on nociceptive transmission. CB₁ receptors inhibit synaptic release of glutamate in rat dorsolateral striatum, a similar mechanism of action may underlie the effects of ACEA on noxious evoked responses of spinal neurons reported here.

INTRODUCTION

Cannabinoids act at cannabinoid CB₁/CB₂ receptors coupled to Gı proteins, which inhibit adenylate cyclase and modulate ion channel conductance (see Pertwee et al. 2001). Synthetic cannabinoids and endocannabinoids, such as anandamide, play an important role in the modulation of nociceptive processing (see Pertwee et al. 2001). Electrophysiological studies have demonstrated that systemic (Hohmann et al. 1999) and spinal (Drew et al. 2000) administration of cannabinoids inhibits noxious evoked responses of spinal neurons. Cannabinoid receptors are located on presynaptic primary afferent fibers and postsynaptic dorsal horn neurons of the spinal cord. Rat dorsal root ganglion (DRG) neurons, predominantly medium- and large-sized neurons, can make cannabinoid receptors and insert them on primary afferent terminals (Hohmann and Herkenham 1999). Recently, we have demonstrated functional CB₁ receptors on adult DRG neurons in culture (Millns et al. 2001). Cannabinoid CB₁ receptors are also located on spinal interneurons (Farquhar-Smith et al. 2000). To date, studies of the effects of cannabinoid agonists have used nonselective agonists, such as HU210 (Drew et al. 2000) and WIN 55,212–2 (Hohmann et al. 1999), which act at CB₁ and CB₂ receptors. These anti-nociceptive effects of cannabinoids are partly blocked by the selective CB₁ receptor antagonist SR141716A (see Pertwee et al. 2001), suggesting central antinociceptive effects of the cannabinoids are mediated by CB₁ receptors.

Recently, a synthetic cannabinoid agonist arachidonyl-2-chloroethylamide (ACEA) with a reported 2000-fold selectivity for the CB₁ receptor compared with CB₂ receptor has been described; ACEA has in vivo activity in mice, producing hypothermia, one of the tetrad of behaviors associated with cannabinoids (Hillard et al. 1999). The effect of this selective CB₁ receptor agonist on nociceptive transmission is unknown. Here, changes in noxious evoked responses of spinal dorsal horn neurons following spinal administration of ACEA in the rat are reported.

METHODS

The techniques used have been described previously (Drew et al. 2000). Extracellular recordings of convergent dorsal horn neurons (mean depth, 784 ± 57 μm) were made with parylene-coated tungsten electrodes (A-M Systems) in anesthetized (1–1.5% halothane in 66% N₂O-33% O₂) Sprague-Dawley rats (200–250 g, n = 16). Neuronal responses to transcutaneous electrical stimulation (3 times C-fiber threshold, trains of 16 stimuli at 0.5 Hz delivered by fine stimulating electrodes made in house) of the peripheral receptive field were recorded, and poststimulus histograms were constructed. Evoked responses were separated and quantified on the basis of latencies: Aβ-fiber: 0–20 ms poststimulus; Aδ-fiber: 20–90 ms; C-fiber: 90–300 ms poststimulus. The remaining late neuronal response (300–800 ms poststimulus), occurring as neurons exhibit hyperexcitability following repetitive stimulation, was taken as the C-fiber-mediated postdischarge response of the neuron. The baseline C-fiber-evoked neuronal responses were calculated as the number of action potentials produced by the first stimulation multiplied by the total number of stimuli (16), this response was termed the nonpotentiated response.
### TABLE 1. Evoked responses of dorsal horn neurons in vivo

<table>
<thead>
<tr>
<th>C-fiber</th>
<th>C-fiber</th>
<th>Aβ-fiber</th>
<th>Aδ-fiber</th>
<th>Non-potentiated Post-discharge</th>
</tr>
</thead>
<tbody>
<tr>
<td>Latency, ms</td>
<td>Threshold, mA</td>
<td>APs</td>
<td>APs</td>
<td>C-fiber, APs</td>
</tr>
<tr>
<td>180 ± 13</td>
<td>1.7 ± 0.1</td>
<td>97 ± 10</td>
<td>112 ± 12</td>
<td>382 ± 69</td>
</tr>
</tbody>
</table>

A comparison of control C-fiber threshold, latency, and magnitude of evoked responses of dorsal horn neurons of rats (Aβ-fiber: 0–20 ms post-stimulus; Aδ-fiber: 20–90 ms; C-fiber: 90–300 ms poststimulus and postdischarge: 300–800 ms poststimulus). Values are means ± SE.

Effects of spinal administration of ACEA (0.5–500 ng/50 μl) on evoked responses of dorsal horn neurons (n = 9) in anesthetized rats (n = 9) were studied. Drug effects were measured at 10-min intervals for 50-min postdrug administration. The ability of preadministration (1 h) of the selective CB1 receptor antagonist SR141716A (0.01 μg/50 μl) to block the effect of ACEA (50 ng/50 μl) was studied in separate groups of rats (n = 7). SR141716A was dissolved in distilled H2O and ethanol (<0.1% ethanol), at this concentration SR141716A alone had no effect (data not shown).

Mean maximal effects of ACEA are presented as percentage of the control response ± SE; statistical analysis was performed with repeated-measures ANOVA and Dunn’s multiple comparison test. Comparison of the effect of ACEA in the presence and absence of SR141716A on evoked responses were made with area under the curve (AUC) analysis (percentage control response vs. time postdrug administration). Statistical analysis of AUCs was performed with the Mann-Whitney test.

### RESULTS

Dorsal horn neurons with peripheral receptive fields on the hindpaw were used in this study. C-fiber thresholds of activation, response latency of C-fiber responses and control A- and C-fiber-evoked responses of dorsal horn neurons were measured (Table 1). An example of a control response of a single neuron and a poststimulus histogram after transcutaneous stimulation are depicted in Fig. 1A.

Spinal administration of ACEA (0.5–500 ng/50 μl) inhibited C-fiber-mediated postdischarge responses of dorsal horn neurons in a dose-related manner (Fig. 1, A–E). Mean maximal effects of 0.5, 5, 50, and 500 ng/50 μl ACEA on the postdischarge responses of neurons were 79 ± 6, 62 ± 10, 54 ± 7% (P < 0.01), 45 ± 6% (P < 0.01), of control, respectively. Spinal ACEA (500 ng/50 μl) also reduced the C-fiber-evoked nonpotentiatesd responses of neurons (59 ± 9% of control, P < 0.05) and Aδ-fiber-evoked responses of neurons (68 ± 10% of control, P < 0.01). By contrast, spinal ACEA had minor effects on Aβ-fiber-evoked responses of dorsal horn neurons (data not shown).

Spinal administration of the selective CB1 receptor antagonist SR141716A (0.01 μg/50 μl) significantly blocked the inhibitory effect of ACEA (500 ng/50 μl) on the C-fiber-mediated postdischarge response of neurons (ACEA: AUC = 2,677 ± 384, ACEA/SR141716A: AUC = 4,626 ± 449, P < 0.05, Fig. 2). Effects of ACEA on the nonpotentiatesd component of the C-fiber-evoked response and Aδ-fiber-evoked responses were also reduced by spinal administration of SR141716A (data not shown).

### DISCUSSION

This study demonstrates that activation of spinal CB1 receptors reduces nociceptive transmission. ACEA selectively inhibited Aβ- and C-fiber-mediated responses of dorsal horn neurons in anesthetized rats; Aβ-fiber-evoked responses were unaffected. These results corroborate our recent report that the nonselective cannabinoid agonist HU210 inhibits noxious evoked responses of dorsal horn neurons and that HU210 stimulates [35S]GTPS binding in spinal cord slices of rats (Drew et al. 2000). The selective CB1 antagonist SR141716A blocked the inhibitory effect of ACEA reported here.

The synaptic sites and cellular mechanisms by which cannabinoids selectively inhibit nociceptive transmission at the level of the spinal cord remains unresolved. Indeed these effects could be mediated by both pre- and/or postsynaptic cannabinoid receptors (see earlier). Cannabinoid receptor-mediated inhibitions of N- and P/Q-type calcium channels have been reported in cultured rat hippocampal neurons (Twitchell et al. 1997). Given the role of N- and P-type calcium channels in nociceptive transmission (Diaz and Dickenson 1997), it is feasible that cannabinoid receptor-mediated inhibition of these channels contributes to the effects of cannabinoid agonists on C-fiber-driven neuronal responses. Evidence suggests that activation of CB1 receptors inhibits synaptic release of glutamate in rat dorsolateral striatum (Gerdesman and Lovinger 2001); a similar mechanism of action may underlie the observed effects of ACEA on noxious evoked responses of dorsal horn neurons reported here.
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REFERENCES


