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Systemic cannabinoids produce CB_1-mediated antinociception by activation of descending serotonergic pathways that act upon spinal 5-HT_7 and 5-HT_{2A} receptors

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ABSTRACT

Serotonin (5-HT) plays an important role in the descending control of pain. We evaluated the role of descending serotonergic pathways and spinal 5-HT_7 and 5-HT_{2A} receptors in comparison to that of 5-HT_{1A} and 5-HT_3 receptors in the antinociceptive effects of systemically administered cannabinoids. Antinociceptive effects were evaluated by radiant heat tail-flick and hot plate tests in Balb-C mice. The selective CB_1 receptor agonist, ACEA; a mixed CB_1 and CB_2 receptor agonist, WIN 55,212-2; and a selective CB_2 receptor agonist, GW405833, were given systemically to induce antinociception. Spinal 5-HT was depleted with intrathecal (i.th.) injection of 5,7-dihydroxytryptamine (5,7-DHT). Bilateral surgical lesions of the dorsolateral funiculus were performed. Selective 5-HT_7, 5-HT_{2A}, 5-HT_{1A} and 5-HT_3 antagonists—SB-269970, ketanserin, WAY 100635 and ondansetron, respectively—were administered i.th. Risperidone, an atypical antipsychotic displaying 5-HT_{2A} antagonism, also irreversibly binds to and inactivates the 5-HT_7 receptors. Thus, we also injected risperidone i.th. to elucidate the role of spinal 5-HT_7 and 5-HT_{2A} receptors in cannabinoid-mediated antinociception. WIN 55,212-2 and ACEA produced dose-dependent antinociception, which were reversed by selective CB_1 receptor antagonist rimonabant. GW405833 did not produce any antinociception. The antinociceptive effects of WIN 55,212-2 and ACEA were totally absent in spinal 5-HT depleted and dorsolateral funiculus lesioned mice. I.th. administration of SB-269970, ketanserin, and risperidone, but not WAY 100635 or ondansetron, blocked both WIN 55,212-2- and ACEA-induced antinociception. These findings suggest that systemically administered cannabinoids interact with descending serotonergic pathways via CB_1-mediated mechanisms and exert a central antinociceptive effect involving spinal 5-HT_7 and 5-HT_{2A} receptors.

1. Introduction

Analgesic drugs are generally administered systemically to treat pain. Cannabinoids are analgesic drugs that produce potent antinociceptive effects through activation of CB_1 and CB_2 receptors in the peripheral, spinal and supraspinal sites (Walker and Huang, 2002; Dogrul et al., 2003; Pertwee, 2006; Svízenská et al., 2008). The demonstration that the antinociceptive effects of systemically administered cannabinoids are reduced following spinal transection supports the notion that descending pain modulatory circuits play an important role in systemic cannabinoid-mediated antinociception (Lichtman and Martin, 1991). CB_1 receptors are found in the cerebral cortex, amygdala, hippocampus, basal ganglia, cerebellum, and brain areas involved in descending pain modulation, such as the periaqueductal gray matter, rostral ventromedial medulla, and spinal cord (Hohmann and Suplita, 2006; Garzón et al., 2009; Svízenská et al, 2008). By contrast, CB_2 receptors are localized predominantly outside of the nervous system (Svízenská et al., 2008). The finding that direct local administration of cannabinoids into the periaquaductal gray or rostraventromedial medulla inhibits nociceptive response suggests that one mechanism by which systemically administered cannabinoids produce antinociception is the activation of descending pain inhibitory pathways (Martin et al., 1995, 1998; Meng et al., 1998; Finn et al., 2003).

The spinal cord dorsal horn neurons are an important site for pain transmission and are subject to descending modulation from supraspinal sites (Chen et al., 2005; Heinricher et al., 2009). It is well known that the spinally projecting brain stem serotonergic neurons are involved in the transmission of acute pain, and that the antinociceptive activity of various analgesics depends on the integrity of descending serotonergic pathways (Millan, 2002; Dogrul and Seyrek, 2006). Although various studies suggest an interaction between the cannabinoid and serotonergic systems in the brain through CB_1 receptor mechanisms (Egashira et al., 2008; Bambico et al., 2007; Lau and Schloss, 2008; Häring et al., 2007), there are few

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studies that explore descending bulbospinal serotonergic pathways in the antinociceptive effects of cannabinoids (Palazzo et al., 2006; Mallet et al., 2008). 5-HT affects nociception via seven families of 5-HT receptors (5-HT_{1-7}) (Millan, 2002). Various studies have suggested particular roles of 5-HT_{1A}, 5-HT_{2A}, 5-HT_3 and 5-HT_7 receptors in descending inhibition of pain (Dogrul et al., 2009; Millan, 2002). Immunocytochemical studies found that 5-HT_7 and 5-HT_{2A} receptors are localized in the superficial layers of the spinal cord dorsal horn, which is consistent with a predominant role of these receptors in the control of nociception (Meuser et al., 2002; Xie et al., 2008; Doly et al., 2004, 2005; Noga et al., 2009).

The present study was undertaken to evaluate the role of descending serotonergic systems and spinal 5-HT_7 and 5-HT_{2A} receptors, compared to 5-HT_{1A} and 5-HT_3 receptors, in the antinociceptive effects of systemically administered cannabinoids. We used a variety of approaches, including the selective denervation of spinal serotonergic neurons, dorsolateral funiculus lesions, and intrathecal administration of selective competitive 5-HT_7, 5-HT_{2A}, 5-HT_{1A} and 5-HT_3 receptor antagonists, to identify descending serotonergic pathways in cannabinoid antinociception. Risperidone, an atypical antipsychotic displaying competetive 5-HT_{2A} and D_2 receptor antagonism, elicits a novel mechanism of antagonism on 5-HT_7 receptors, irreversibly binding to and inactivating them (Smith et al., 2006; Toohey et al., 2009). Thus, we also used risperidone to examine the contribution of spinal 5-HT_7 and 5-HT_{2A} receptors in the antinociceptive effects of systemically administered cannabinoids.

2. Materials and methods

2.1. Animals

Adult male Balb-C mice (25–30 g) were used. The mice were housed in a room maintained at 22 ± 3 °C and 50–55% humidity on a 12-h. light–dark cycle (lights on at 08:00 a.m.). All experiments were conducted in accordance with the guidelines set forth by the International Association for the Study of Pain (Zimmermann, 1983).

2.2. Drugs

R-(+)-WIN 55,212-2 (R(+)-[2,3-Dihydro-5-methyl-3[(morpholinyl) methyl] pyrolo[1,2,3-de]-1,4-benzoxazinyl]-(1-naphthalenyl) methanone mesylate (Sigma-RBI, USA), a mixed CB_1/CB_2 receptor agonist and rimonabant (kindly provided by Sanofi-Aventis, France), a selective CB_1 receptor antagonist, were dissolved in a 20:1:1:78 (v/v/v/v) mixture of DMSO: ethanol: Tween 80: 0.9% physiologic saline. ACEA (Arachidonyl-20-chloroethylamide) (Tocris, UK), a selective CB_1 agonist with a reported 2000-fold selectivity for the CB_1 receptor compared with the CB_2 receptor (Hillard et al., 1999), was suspended in 40:30:30 (v/v/v) mixture of ethanol: DMSO: 0.9% physiologic saline. GW405833 (2,3-dichloro-phenyl)-[5-methoxy-2-methyl-3-(2-morpholin-4-yl-ethyl)-indol-1-yl]-methanone) (Tocris, UK), a potent and selective CB_2 receptor agonist with a reported 1200-fold selectivity over the CB_2 receptor compared with the CB_1 receptor (Valenzano et al., 2005), was dissolved in 25% hydroxypropyl-β-cyclodextrin in distilled water.

Cannabinoid agonists were administered intraperitoneally (i.p.) in a volume of 5 ml/kg. Rimonabant was given 20 min prior to administration of WIN 55,212-2 and ACEA. The SB-269970 (2R-1-[(3-hydroxyphenyl)sulfonyl]-2-[2-(4-methyl-1-piperidinyl)ethyl]-pyrrolidine) hydrochloride, which is reported to have a selective 5-HT_7 antagonist displaying over 250-fold selectivity versus other 5-HT receptors (Lovell et al., 2000); a selective 5-HT_{1A} receptor antagonist, WAY 100635 8N-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl-N-(2-pyridinyl)cyclohexanecarboxamide trihydrochloride) maleate (Forster et al., 1995); and a dopamine D_2 receptor antagonist, chlorpromazine hydrochloride, were obtained from Sigma-RBI (USA). A 5-HT_{2A} antagonist, ketanserin tartrate, was obtained from RBI (USA). Ondansetron, a highly selective 5-HT_3 receptor antagonist with a selectivity ratio of approximately 1000:1 compared with affinities for other receptors (Freeman et al., 1992), was obtained by GlaxoSmithKline (Zofran, Turkey). Risperidone was kindly provided by Johnson & Johnson. 5,7-DHT, SB-269970, ketanserin, WAY 100635 and chlorpromazine were freshly dissolved in 0.9% saline prior to injection. Risperidone was dissolved in 25% DMSO. Fixed doses of SB-269970 (10 μg), risperidone (10 μg), ketanserin (10 μg), chlorpromazine (10 μg), WAY 100635 (10 μg) and ondansetron (10 μg) were given i.th. 30 min after the i.p. administration of different doses of WIN 55,212-2 and ACEA. These spinal doses of 5-HT antagonists were chosen from our previous studies (Dogrul and Seyrek, 2006; Dogrul et al., 2009).

2.3. Assessment of nociception

The radiant heat tail-flick test (Columbus, OH, USA; Type 812) and hot plate tests (MAY 9610, Commat Ltd, Turkey) were used to assess nociception. For the radiant tail-flick test, the intensity of the beam was adjusted to produce mean control reaction times of 2–3 s. The hot plate test apparatus consisted of an electrically heated surface kept at a constant temperature of 52.0 ± 0.6 °C. Baseline tail-flick and hot plate latencies for each mouse were determined before treatment; test latencies were measured after the drug injections. The latencies for paw licking or jumping were recorded for each animal for hot plate test. A cut-off time of 6 s and 60 s were used to prevent tissue damage for tail-flick and hot plate tests, respectively.

I.th. injections were performed according to the method of Hylden and Wilcox (1980). A 30 G needle was inserted into the lumbar space between the L5 and L6 vertebrae of unanesthetized mice and a volume of 10 μl was injected. Control animals received vehicle as control. Dose–response curves were constructed from data gathered 30 min after the i.th. injection of 5-HT receptor antagonists or 60 min. after i.p. administration of WIN 55,212-2 and ACEA. The data were converted to % Analgesia by the formula % Analgesia = 100 × (Test latencies − baseline latencies) / (Cut-off latency − baseline latencies) in dose–response curves.

2.3.1. 5,7-DHT administration

Endogenous spinal 5-HT was depleted with the neurotoxin 5,7-DHT (creatinin sulphate salt, Sigma-USA). The mice were pretreated with desipramine hydrochloride (25 mg/kg) (i.p.) to prevent uptake of 5,7-DHT into noradrenergic terminals. After 45 min., mice were given either i.th. 0.9% saline or 50 μg of 5,7-DHT. On the fourth day following 5,7-DHT administration, behavioral tests were performed. Our recent and previous studies have reported that this procedure and the i.th. dose of 5,7-DHT reduced endogenous spinal 5-HT contents by more than 88% in mice four days after treatment (Yanarates et al., 2010; Hung et al., 2003).

2.4. Dorsolateral funiculus lesion

The previously described microforceps compression technique for dorsolateral funiculus lesioning in rats is not applicable for mice because of the small diameter of the spinal cord. For this reason, we employed a microknife instead of microforceps. Under anesthesia with ketamine (80 mg/kg) and xylazine (7 mg/kg) (i.p.), dorsolateral funiculus lesions were made at the T8–10 level.

The skin at T8–10 level was incised and a laminectomy was performed under a surgical microscope. The dura mater was cut open with iris scissors and a 0.1 ml 2% lidocaine solution was applied to the dorsal surface of the cord. The dorsolateral part of the spinal cord lateral to the dorsal root entry zone, between two segmental dorsal roots, was identified under the surgical microscope with ×40 magnification. A sagittal cut using a micro-arachnoid knife for