Published in final edited form as:

Psychopharmacology (Berl). 2011 August ; 216(3): 323–331. doi:10.1007/s00213-011-2224-5.

CB₁ receptors mediate rimonabant-induced pruritic responses in mice: investigation of locus of action

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Abstract

Rationale—Cannabinoids have recently been identified as potential neuronal modulators of pruritic response, representing a potential target in the treatment of itch associated with a variety of pathophysiologic conditions. While the selective CB₁ receptor antagonist rimonabant is an established pruritic agent in both animal and clinical testing, its receptor mechanism of action and anatomical loci remain unclear.

Objective—The purpose of this study was to determine whether CB₁ receptor blockade is critical to rimonabant-induced scratching and to identify differences in scratching response based on different routes of administration. Furthermore, experiments were designed to elucidate any evidence as to whether rimonabant elicits scratching behavior through common immunologic hypersensitivity mechanisms.

Results—Rimonabant was equally effective at producing scratching via intraperitoneal and local subcutaneous injection. This compound also produced an intense scratching response when administered intrathecally, but had no effects after intracerebroventricular administration. Repeated administration of rimonabant led to a decreased magnitude of scratching. While rimonabant-induced scratching was not attenuated either by pretreatment with the H₁ receptor antagonist loratadine or in mast cell-deficient mice, it lacked efficacy in CB₁ (–/–) mice.

Conclusions—Rimonabant is a potent and fully effective pruritogen when administered spinally or systemically and requires CB₁ receptors to induce scratching, suggesting an important spinal CB₁ receptor component of action. The lack of responsiveness to H₁ antagonism or mast cell deficiency supports previous findings that cannabinoids modulate itch through neuronal mechanisms, and not by traditional hypersensitivity activation.

Keywords

Cannabinoid; Rimonabant; Compound 48/80; CB₁; Intrathecal; Intracerebroventricular; Itch; Scratching; Pruritus; Mast cell

Introduction

Recent studies in the area of pruritus, the general clinical term for itch, have built a new model of how this sensory process works. Importantly, clear evidence suggests that while the induction of itch is predominantly a function of immune response, the transmission and

perception of itch is a specialized and distinct neuronal process. While likely transmitted by similar spinothalamic pathways as those of nociception or pain sensory information, pruritic afferent signals appear to be carried via distinct and now potentially distinguishable neural tracts (Andrew and Craig 2001; Sun et al. 2009). Furthermore, extensive clinical studies suggest that these two similar sensory modalities may in fact operate competitively when activated simultaneously (Ward et al. 1996; Yosipovitch et al. 2007; Yosipovitch et al. 2005). This expanding area of research may soon establish a clear neurobiological schematic of how we perceive itch, regardless of the original physical insult. Ablation of specific neurons containing the gastrin-releasing peptide receptor that was implicated in neuronal activation during pruritus prevented scratching responses to a wide variety of pruritic insults thought to work via divergent mechanisms (Sun et al. 2009). With this sort of information, the question will now become why there are no treatments necessarily designed to target intractable itch at the neuronal level. Most common treatments of itch, such as antihistamines and corticosteroids, are meant to prevent any initiation of itch by immune mediators. For itch not associated with classical immune mediators at the level of the skin, any number of drugs are attempted to treat the symptoms, many being traditional pain medications. This new line of research may lead to a more specific class of drugs that can be used as a second line of treatment.

In a recent paper, we demonstrated that increasing the endocannabinoid anandamide, via inhibition of the degradative enzyme fatty acid amide hydrolase (FAAH), could reduce allergenic scratching induced by the common experimental pruritogen and mast cell degranulator compound 48/80. Mechanistic examination of this phenomenon led to evidence that the anti-scratching effects were produced via CB₁ receptor activity and FAAH present in neuronal tissue (Schlosburg et al. 2009). This work added to previous findings that non-selective CB₁/CB₂ cannabinoid agonists could reverse scratching responses to the serotonergic agonist 2,5-dimethoxy-4-iodoamphetamine in mice (Darmani 2001) and suppress itch sensation in humans by localized application of histamine (Dvorak et al. 2003).

It is firmly established that systemic administration of rimonabant, the CB₁ receptor antagonist, induces increases in scratching, head twitch, and grooming behaviors in rats and mice (Cook et al. 1998; Rubino et al. 2000; Tallett et al. 2007; Vickers et al. 2003). Further studies in mice established that mixed CB₁/CB₂ receptor agonists fully reversed scratching and head twitch behavior (Janoyan et al. 2002), while 5-HT_{2A/C}, AMPA/kainite, and NK₁ receptor antagonists partially reversed these effects (Darmani and Pandya 2000). However, these behaviors are differentially sensitive to the aforementioned antagonists, suggesting potentially distinct mechanisms for their induction by rimonabant. Rats show a developmental subsiding of scratching intensity from youth to adulthood, with scratching largely reversed via 5-HT_{2A/C} receptor antagonism (Ward et al. 2009). Most of the receptors implicated in the studies conducted to date are G-protein-coupled receptors that are commonly associated with neuronal activity, especially in the modulation and transmission of sensory information, while rimonabant itself is highly potent and selective for the CB₁ receptor (Rinaldi-Carmona et al. 1994). Information from the clinical use of rimonabant as a weight loss medication for obese patients suggests that itching was a common side effect reported by 1–10% of patients (European Medicines Agency 2008). Thus, clinical data demonstrate bidirectional control of itch response via CB₁ receptor agonists and antagonists. The primary objective of the present study was to determine the underlying mechanisms of rimonabant-induced scratching. To this end, we first evaluated the pruritic actions of rimonabant via systemic and central routes of administration. We then assessed whether rimonabant elicited scratching behavior in CB₁ (–/–) mice, mast cell deficient mice, and wild-type mice treated with the H₁ receptor antagonist, loratadine.

Methods

Subjects

Male and female CB₁ (–/–) mice and their littermate controls were obtained from the Center Transgenic Colony at Virginia Commonwealth University (Richmond, VA) backcrossed onto a C57BL/6J (13 generations) background. Male *W^{sh}/W^{sh}* mutant mice, an established line of mast cell-deficient mice, were also bred at Virginia Commonwealth University on a C57BL/6J background. All other experiments utilized adult male C57BL/6J mice (C57; The Jackson Laboratory, Bar Harbor, ME). Mice were housed 4–6 per cage in a temperature (20–22°C)-controlled environment with food and water available ad libitum. Mice were kept on a 12-h light/dark cycle, with all experiments performed during the animals' light period. All experiments were performed with the approval of the Institutional Animal Care and Use Committee at Virginia Commonwealth University in accordance with the Guide for the Care and Use of Laboratory Animals.

Drugs and injections

Rimonabant and Δ^9 -tetrahydrocannabinol (THC) were obtained from the National Institute on Drug Abuse (Bethesda, MD) and were dissolved in a vehicle consisting of ethanol/alkamuls-620 (Rhone-Poulenc, Princeton, NJ)/saline in a ratio of 1:1:18. Compound 48/80 and loratadine were purchased from Sigma (St. Louis, MO) and dissolved in 0.9% saline. All drugs were diluted to an injection volume of 10 μ L/g body mass when administered via intraperitoneal injection, while compound 48/80 was administered as a fixed quantity diluted per 100 μ L subcutaneous injection, given at the scruff of the neck. Drugs administered via intrathecal and intracerebroventricular injections were concentrated to be administered using the 1:1:18 vehicle at a fixed volume of 5 μ L/injection. As noted below, a single group was given intracerebroventricular injections of rimonabant in a 1:1:18 vehicle substituting DMSO for ethanol. Intrathecal injections were performed according to the protocol of Hylden and Wilcox (1983), in which naïve mice were injected at the L5/6 region with a 30-gauge needle. Intracerebroventricular (icv) injection was performed using a modified protocol from Pedigo et al. (1975), in which isoflurane anesthetized mice had an incision made in the scalp in order to expose bregma. Injections were performed using a 26-gauge needle, with a sleeve of PE20 tubing to control the depth of the injection, at a site 2 mm rostral and 2 mm caudal to the bregma and at a depth of 2 mm. Pilot studies injecting dye into anesthetized mice established that the injections were infused in the ventricles almost 100% of the time.

Behavioral evaluation of scratching response

The testing apparatus used in monitoring scratching consisted of a white (for contrast) acrylic box (20 × 20 × 20 cm), with a clear acrylic front panel and a mirrored back panel. The chambers were enclosed in sound-attenuating cabinets, designed and custom built at Virginia Commonwealth University, that contained an indirect filtered LED light source and fans for air circulation and white noise. Mice were placed in the boxes for 30 min to acclimate to the environment. The mice were then briefly removed from the boxes, which were wiped clean with water and dried. Subjects were then given an injection of a pruritic compound (i.e., rimonabant or compound 48/80) or vehicle, and were returned to the boxes for a recording period of 60 min following rimonabant or 30 min following compound 48/80. Behavior was recorded through the clear front panel using a series of Fire-i™ digital cameras (Unibrain, San Ramon, CA) and the videos were processed and saved using ANY-maze™ video tracking software (Stoelting Co., Wood Dale, IL). Chambers were fully sanitized at the end of each testing day using ammonia-based cleansers and soap, then left to air dry at least 2 days to dissipate any odors.

The videos were subsequently placed in randomized order in a separate ANY-maze™ protocol for a trained observer to score using a keyboard-based behavioral tracking system, blinded to treatment group. Either ANY-maze™ or ODLog™2 (Macropod Software, Armidale, NSW, Australia) software was used to track key presses assigned to specific behavioral end points for both time pressed and number of occurrences. The scratching response was tracked as hind leg scratching of the animal's sides, dorsal torso, and head, while excluding behaviors focused at areas within the ears or flicking and pulling at ear tags. Behavioral locomotor inactivity was also monitored and scored as complete lack of voluntary movement for any continuous period longer than 5 s.

Evaluation of cannabinoid antinociception and hypothermia

Animals were evaluated for baseline antinociception and hypothermia, followed by administration of either icv vehicle or rimonabant. Animals were tested again following a 5-min recovery period for any changes due to icv injection, and then injected with either vehicle or THC (50 mg/kg, i.p.). The final set of nociceptive and temperature measurements were taken 15 min following THC injection.

Subjects were assessed for nociception in the tail immersion assay. Each mouse was placed head first into a small bag fabricated from absorbent under pads (VWR Scientific Products; 4 cm diameter, 11 cm length) with the tail out of the bag. The experimenter gently held the mouse and immersed approximately 1 cm of the tip of the tail into a water bath maintained at 52.0°C. The latency for the animal to withdraw its tail was timed, with a 10-s cutoff to prevent tissue damage. The tail withdrawal data were expressed as a percentage of maximal possible effect [%MPE=post-THC latency–pre-THC latency)/(10-s cutoff–pre-THC latency)]. Rectal temperature was determined by inserting a thermocouple probe 2.0 cm into the rectum, and temperature was obtained from a BAT-10 digital telethermometer (Physitemp Instruments, Inc., Clifton, NJ).

Data analysis

All data are reported as mean±SEM and represent the total number of seconds a specific behavior was scored from a total observational period of 1,800 s, summed from 5-min bins. Experiments with only two treatment groups were analyzed for statistical significance using Student's *t* test. Experiments with more than two groups were analyzed using one-way, two-way, or multifactorial repeated measures analysis of variance, with specified appropriate post hoc test. Resulting *p* values of less than 0.05 were considered significant.

Results

Dose–response and localization of rimonabant scratching

Our initial experiments involved evaluating the levels of spontaneous scratching elicited by rimonabant via systemic and central routes of administration. Vehicle administration alone produced similar levels of spontaneous scratching as that seen in naïve, untreated mice. Intraperitoneal administration of rimonabant elicited a dose-dependent increase in scratching behavior [$F(3, 33)=5.5$, $p<0.01$] directed at the sides and back of the torso, as well as the head and ears (Fig. 1a). The time spent scratching was significantly increased from vehicle at 10 and 30 mg/kg. Rimonabant (30 mg/kg) administered subcutaneously beneath the scruff elicited similar levels of scratching as when given via i.p. administration (Fig. 1a). None of these treatments were associated with altered levels of general inactivity ($p>0.05$). The incidence of head twitch behavior was noted during our observations, often occurring at the culmination of a prolonged period of scratching. However, the number of twitches was not quantified in the present study.

When given directly into the subarachnoid space of the spinal column, rimonabant also dose-dependently increased scratching [$F(3, 24)=12.8$, $p<0.001$] to equivalent levels as those seen following systemic injection (Fig. 1b). Injections of 5 μ l containing 18 or 36 μ g of rimonabant (in 1:1:18 vehicle) elicited significant increases in scratching behavior, without any detrimental changes in general cage activity ($p>0.05$). The noted observations of head twitch behavior following intrathecal administration was far lower, with only about a third of mice showing any twitch behavior subsequent to rimonabant injection.

We next evaluated whether rimonabant (36 μ g in 5 μ l of 1:1:18 vehicle) administered via icv injection would elicit scratching behavior. A preliminary study was conducted to confirm whether this dose of rimonabant was bioactive by testing whether pretreatment would block the antinociceptive and hypothermic effects of THC (50 mg/kg, i.p.). Mice given an icv injection of rimonabant showed approximately half the antinociceptive behavior [$t(9)=3.0$, $p<0.01$] (Fig. 2a) and virtually no hypothermia [$t(9)=5.7$, $p<0.001$] (Fig. 2b) following a high dose of THC. Rimonabant, icv, did not elicit either scratching [vehicle, 0.2 ± 0.1 s; rimonabant, 0.0 ± 0.0 s] or reduce open field activity [vehicle immobility time, 125.3 ± 62.4 s; rimonabant immobility time, 232.2 ± 72.7 s; $p=0.28$]. Due to concerns that inactivity was associated with ethanol content in the vehicle, the study was repeated using a DMSO-based vehicle, but yielded a similar pattern of minimal scratching behavior [vehicle, 15.6 ± 7.3 s; rimonabant, 9.8 ± 4.2 s].

CB₁ receptor dependence in rimonabant scratching

Previous reports have already established that mixed CB₁/CB₂ agonists can dose-dependently reverse the scratching behavior elicited by rimonabant and in similar order of potency as their CB₁ receptor binding affinity. However, the doses required to do so typically corresponded with doses that also inhibited spontaneous activity and general cage behavior (Janoyan et al. 2002). In order to determine whether the CB₁ receptor was necessary for rimonabant-induced scratching, we recorded the duration of time spent scratching in CB₁ (+/+) and (-/-) mice treated with rimonabant. Given the established sensitivity to increased skin irritation and hair loss associated with older CB₁ (-/-) mice (Karsak et al. 2007; Vaccarino et al. 1985), young adult mice were used, and the mice were monitored for scratching behavior 30 min prior to rimonabant administration (10 mg/kg, i.p.). While no significant differences were observed prior to injection, CB₁ (+/+) mice almost immediately began scratching upon rimonabant injection, while CB₁ (-/-) mice failed to show significant levels of scratching behavior [Genotype $\times$ time, $F(8, 80)=4.5$; $p<0.01$] (Fig. 3a). When compiling the total time scratching (Fig. 3b) over the hour following rimonabant, CB₁ (+/+) mice scratched to similar levels reported above, while CB₁ (-/-) mice showed levels similar to baseline behavior [$t(10)=7.0$; $p<0.001$].

Tolerance to scratching effects following repeated rimonabant

Next, we examined if the repeated administration of rimonabant scratching undergoes tolerance. Pruritic secretagogues (drugs that induce degranulation of immune cells involved in hypersensitivity reactions) can often deplete mast cells and basophils of histamine and serotonin upon repeated administration, causing near complete loss of scratching behavior. Following 6 days of daily treatment with rimonabant (10mg/kg), mice still scratched significantly more than mice receiving vehicle, but for a significantly shorter period of time than that of acute exposure [$F(2, 15)=36$, $p<0.001$]. Repeated rimonabant resulted in approximately half the original pruritic response (Fig. 4).

Rimonabant scratching following alterations to immunologic response

As rimonabant appears to be operating via spinal CB₁ receptors, we examined if the scratching response is also mediated by common allergenic hypersensitivity mechanisms,

typically operating at the level of the skin. We first examined whether rimonabant (10 mg/kg, i.p.) scratching would be altered by pretreatment with the second-generation H₁ antagonist loratadine (10 mg/kg, i.p.). Unexpectedly, rimonabant scratching time was unresponsive to antihistamine treatment (Fig. 5a). This finding differs from the effects produced by the allergenic pruritogen and mast cell degranulator compound 48/80 (30 µg, s.c.), in which scratching behavior was reduced by roughly half following antihistamine pretreatment [Pruritogen×treatment, $F(1, 20) = 4.7$, $p < 0.05$].

While the failure of loratadine to attenuate rimonabant-induced scratching suggests the lack of involvement of typical hypersensitivity mechanisms, serotonin, not histamine, is the major component of mast cells in mice (Granberg et al. 2001). Since serotonin works at numerous sites of action within the sensory tract, we chose to examine scratching response in W^{sh}/W^{sh} mice, which are an established in vivo model of mast cell deficiency. Homozygous mutant mice exponentially lose mast cell content from birth and have relatively few mast cells at adulthood, less than 1% as compared to C57 wild-type controls (Yamazaki et al. 1994). We first examined their ordinary scratching behavior after simple saline injection and found similar spontaneous scratching between genotypes. We also found W^{sh}/W^{sh} mice similarly responsive to rimonabant as C57 controls. Interestingly, W^{sh}/W^{sh} mice also displayed wild-type levels of scratching to the mast cell degranulator compound 48/80 (Fig. 5b). Thus, while compound 48/80 is immune-mediated, neither pruritogen requires the presence of mast cells in skin or the surrounding tissues.

Discussion

This study represents the first direct evidence that CB₁ receptors are necessary to produce rimonabant-induced scratching. While this finding is not entirely surprising, given the highly potent and selective nature of rimonabant's binding profile for CB₁ receptors, it confirms that inactivation or inverse agonism at these receptors can result in immediate induction of scratching behavior. Systemic administration of rimonabant resulted in significantly increased scratching, but typically at doses well above those required to block the effects of exogenous cannabinoids (Rinaldi-Carmona et al. 1994). Consistent with our previous findings (Schlosburg et al. 2009), as well as those of others (Darmani and Pandya 2000; Janoyan et al. 2002), doses below 1 mg/kg did not elicit spontaneous scratching behavior. It is plausible that rimonabant triggers scratching behavior because it antagonizes endogenous cannabinoids at the receptor. Conversely, the fact that high doses of rimonabant are required to elicit scratching is harmonious with the possibility that the effect is mediated via inverse agonism of CB₁ receptors and concomitant decreases in inhibitory G_{i/o} basal activity (Landsman et al. 1997; Sim-Selley et al. 2001). However, current reports using a purported neutral CB₁ antagonist show similar scratching behavior induced by systemic administration (Jarbe et al. 2008). Furthermore, when examining the doses of rimonabant that induce scratching when injected into the spinal column, they closely match those required to reverse cannabinoid antinociception (Welch et al. 1998), suggesting that direct spinal CB₁ receptor inhibition may be more sensitive and may lead to scratching behavior without excessive dosing. That we were able to induce the same magnitude of scratching response by direct administration into the spine as observed following systemic administration promotes the likelihood that spinal CB₁ receptors are largely responsible for the modulation of scratching by cannabinoids.

While the precision of intrathecal and icv injections at moderate volumes can be subject to rostral or caudal diffusion, our findings indicate differential effects between these two types of injections. The high dose of icv rimonabant completely blocked THC-induced hypothermia and partially blocked THC-induced antinociception but failed to elicit scratching. This differential effectiveness of rimonabant can be attributed to these end points

being mediated by supraspinal versus spinal and peripheral loci of action. Cannabinoid-induced hypothermia is predominantly mediated by the preoptic area of the hypothalamus (Fitton and Pertwee 1982; Schmeling and Hosko 1980), which is adjacent to the third ventricle and is consistent with our observations shown in Fig. 2b. Our finding that icv rimonabant only partially blocked THC-induced antinociception is not surprising given that cannabinoids activate antinociceptive signaling pathways at multiple levels of the neural axis, including multiple brain regions, dorsal horn of the spinal cord, and afferent nerves in the periphery (Kelly and Donaldson 2008; Richardson et al. 1998). These results confirm that we are effectively administering an active dose of rimonabant into the cerebral ventricles; however, we were unable to observe scratching behavior as a result. We did observe a reduced level of activity in mice administered icv injections regardless of treatment, and the procedure is far more invasive than intrathecal injection, but using a different vehicle did not alter results and mice generally regained normal activity levels within 10 min using either ethanol or DMSO containing vehicle. These data suggest that rimonabant does not elicit pruritic signaling through a direct action in the brain, but at the spinal level, and possibly in the periphery, though activation of supraspinal CB₁ receptors may play a role in attenuating scratching response to other stimuli.

Interestingly, the pruritic effects of rimonabant underwent tolerance following repeated dosing, though significantly increased scratching was still observed. This observation of partial tolerance has also been reported in rats (Vickers et al. 2003). These data also suggest that rimonabant is likely not acting as a secretagogue, such as compound 48/80, as they typically undergo near complete tolerance in multiple models as a result of mast cell depletion (Allansmith et al. 1987; Ambrus and Thurber 1975; Ohtsuki et al. 1985). Further evidence that rimonabant elicits scratching through non-hypersensitivity mechanisms is its unresponsiveness to an H₁ receptor antagonist. In contrast, loratadine partially reversed compound 48/80-induced scratching, in line with evidence that compound 48/80 elicits pruritic responses through the release of both histamine and serotonin from mast cells. However, in an attempt to confirm this mechanism, we found that the *W^{sh}/W^{sh}* mast cell-deficient mice displayed wild-type levels of scratching behavior to both rimonabant and compound 48/80. This is the first report of compound 48/80 maintaining pruritic effects in the *W^{sh}/W^{sh}* model, but matches previous findings that compound 48/80 produced scratching in the more established *W/W^v* mast cell-deficient model. This same report also confirmed that compound 48/80 was, in fact, producing histamine release and mast cell degranulation in ordinary BALB/c mice, but demonstrated that it may not necessarily be the cause of the scratching behavior (Inagaki et al. 2002). Given that compound 48/80-treated mice exhibit reduced scratching behavior after treatment with non-sedative antihistamines (Schlosburg et al. 2009; Sugimoto et al. 1998), the next most likely physiologic mediator would be basophils, for which selective models of ablation have recently been developed (Wada et al. 2010). These data in opposition of established models of itch further underscore the necessity of the current renewed effort in understanding the neurobiology of itch. While our evidence suggests that rimonabant is not acting through the classical hypersensitivity mechanisms, we cannot yet even establish with certainty how the prototypical laboratory allergenic agent specifically promotes pruritic signaling.

Despite the large number of other receptor systems that could potentially mediate rimonabant-induced scratching, the CB₁ (–/–) mouse study indicates that these alternate signaling systems are not influenced by nonspecific effects of rimonabant, but rather interactions with CB₁ receptors to influence neuronal excitability. All studies to date examining the impact of serotonergic and other neurotransmitters on rimonabant scratching have relied on systemic administration of both rimonabant and other antagonists. However, an elegant study demonstrated that systemic rimonabant led to altered brain levels and turnover of serotonin and dopamine in shrews (Darmani et al. 2003). Additional studies in

rostral brain regions show that cannabinoid receptor antagonism increases levels of serotonin (Tzavara et al. 2003) while acute cannabinoid agonist administration reduces levels of serotonergic precursors (Moranta et al. 2009). Systemic CB₁ receptor activation even reduces levels of circulating serotonin in blood (Rutkowska and Gliniak 2009). In addition to serotonin levels, electrophysiological recording in the inferior olive showed that treatment with a cannabinoid receptor antagonist counteracted suppression of synaptic activity mediated by 5-HT₂ receptors (Best and Regehr 2008). Despite extensive and growing knowledge of cannabinoid interactions with serotonergic circuits in the brain, no studies to date have examined their interactions at the level of the dorsal root ganglion (DRG). It will be essential to follow-up on established knowledge with targeted studies to determine exactly where the cannabinoid and serotonergic systems interact to influence scratching responses. A similar study has already suggested that 5-HT₇ and 5-HT_{2A} receptors may be critical to cannabinoid antinociceptive signaling in the spinal cord (Seyrek et al. 2010).

While predominant evidence suggests an interaction between cannabinoid and serotonergic receptors in rimonabant scratching, other systems including tachykinin and opioid systems also have established interactions with CB₁ receptor signaling (Haller et al. 2008; Zhang et al. 2010) and are critical to pruritic signaling in the spinal cord. Consistent with the work of Zhang et al. (2010), it is plausible that rimonabant-induced scratching is mediated via the release of substance P in the spinal cord. Acting in a similar fashion as proteinase-activated receptor 2 receptors, CB₁ receptors may act as presynaptic modulators of neurotransmitter release within the DRG (for review and schematic diagram see Cevikbas et al. 2010). Based on our current findings, spinal CB₁ receptors appear to play a predominant role in rimonabant-induced pruritic responses, though peripheral CB₁ receptors may also contribute. Combined with our previous findings (Schlosburg et al. 2009), CB₁ receptors represent a bidirectional neuronal target for mediating pruritic signaling.

Acknowledgments

The work was supported by P01DA009789 P50DA005274, RO1AI18697, and R01DA015197. Training of author JES was supported by National Institute of Drug Abuse grant F31DA026279 and T32DA007027.

Abbreviations

FAAH	Fatty acid amide hydrolase
Rimonabant	<i>N</i> -(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1 <i>H</i> -pyrazole-3-carboxamide HCl
THC	Δ ⁹ -tetrahydrocannabinol
ANOVA	Analysis of variance
icv	Intracerebroventricular
C57	C57BL/6J mouse strain
DRG	Dorsal root ganglion

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Fig. 1.

Spontaneous scratching is increased as a result of systemic or spinal administration of rimonabant. **a** Dose-response of time spent hind leg scratching induced by intraperitoneal rimonabant and comparison to subcutaneous injection and no injection (naïve). **b** Dose-response of time spent scratching elicited by intrathecal injection of rimonabant. Values represent mean±SEM time scratching within 30 min post-injection. (*Single asterisk*) $p < 0.05$, (*double asterisk*) $p < 0.01$ versus vehicle (Dunnett's post hoc); $n = 8-10$ mice per dose

Fig. 2.

Intracerebroventricular pretreatment with rimonabant partially reverses antinociception (**a**) and fully blocks hypothermia (**b**) associated with systemic THC administration. Rimonabant (30 μ g, icv) or vehicle was given 5 min prior to baseline measurements and immediately followed with an i.p. dose of THC (50 mg/kg). Testing for changes in tail immersion latency and body temperature were repeated 15 min later. Values represent mean $\pm$ SEM. (*Double asterisk*) $p < 0.01$, (*triple asterisk*) $p < 0.01$ versus vehicle (Student's t test); $n = 6$ mice per group

Fig. 3.

Rimobant scratching behavior is absent in CB₁ receptor null mice. **a** Comparative timeline of scratching behavior observed 30 min prior and 1 h following acute injection of rimobant (10 mg/kg, i.p.). CB₁ (+/+) mice show elevated scratching behavior over most of the observed period, **b** resulting in a cumulatively substantial increase above that of CB₁ (-/-) littermates over the 1-h period. Values represent mean±SEM. (Double asterisk) $p < 0.01$, (triple asterisk) $p < 0.001$ versus CB₁ (-/-) group (a Tukey's post hoc, b Student's t test); $n = 6$ mice per group

Fig. 4.

Rimonabant-induced scratching is reduced following repeated injection. Mice were given either 6 days of daily vehicle, 5 days of vehicle, and an acute injection of rimonabant or 6 days of rimonabant (10 mg/kg). Scratching was monitored following injections on the sixth day. Values represent mean $\pm$ SEM. (*Double asterisk*) $p<0.01$, (*triple asterisk*) $p<0.01$ versus vehicle group, (*double number sign*) $p<0.01$ versus acute rimonabant (Tukey's post hoc); $n=6$ mice per group

Fig. 5.

Rimonabant-induced scratching is not responsive to antihistamine treatment or mast cell deficiency. **a** Unlike a known mast cell and basophil degranulator, compound 48/80 (30 μ g, s.c.), rimonabant (10 mg/kg, i.p.) scratching is not reduced by the non-sedative H_1 antagonist loratadine (10 mg/kg, i.p.). **b** Neither rimonabant nor compound 48/80 scratching is reduced in the W^{sh}/W^{sh} skin mast cell-deficient mouse line. (Triple asterisk) $p < 0.01$ versus respective saline group (Tukey's post hoc); $n = 6$ mice per group