



Revisiting the complex influences of cannabinoids on motor functions unravels pharmacodynamic differences between cannabinoid agonists

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ABSTRACT

While numerous cannabinoid ligands were historically characterized using the tetrad test (hypomobility, catalepsy, hypothermia, analgesia), only few studies have extensively compared HU 210 and CP 55,940 which are nowadays classically used as reference agonists. Therefore, we herein re-examined the acute and the sustained changes in motor activities mediated by these two agonists in adult rats. As expected for cannabinoid agonists, exposure to either HU 210 or CP 55,940 induced a marked reduction in spontaneous locomotion. This reduction observed as early as 15 min after injection was correlated with the typical rearing and cataleptic responses, and was reversed by co-administration of the CB₁ cannabinoid receptor antagonist SR 141716A. Nevertheless, HU 210, but not CP 55,940, was found to induce persistent responses, lasting for at least 24 h. Also suggesting the involvement of additional targets for HU 210, 10 mg/kg SR 141716A failed to reverse the persistent HU 210-mediated decline in locomotion and rearing, while 1 mg/kg was sufficient to completely abolish the behavioural responses measured 6 h after the injection. Beside pharmacokinetic differences, these data therefore denote distinct pharmacodynamic profiles for HU 210 and CP 55,940. Together, these results suggest that HU 210 displays multicomponent responses that should be taken into account when interpreting data from *in vivo/ex vivo* studies.

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1. Introduction

Since the cloning of the cannabinoid receptors and the discovery of endogenous cannabinoid ligands in the early 1990s, extensive research has revealed the involvement of the cannabinoid system in several physiological processes including the modulation of neurotransmission, the regulation of energy homeostasis and the control of immune cell functions (Pacher et al., 2006). These events are mainly mediated through activation of two cannabinoid receptors, the CB₁ and the CB₂ cannabinoid receptors, which are currently recognised as the key cannabinoid targets. As members of the G protein-coupled receptor superfamily, both cannabinoid receptor subtypes were reported to exert their effects through activation of Gi/o-type G proteins (Howlett et al., 2002).

Consistent with a widespread distribution in the brain, a variety of behavioural responses are triggered upon activation of the CB₁

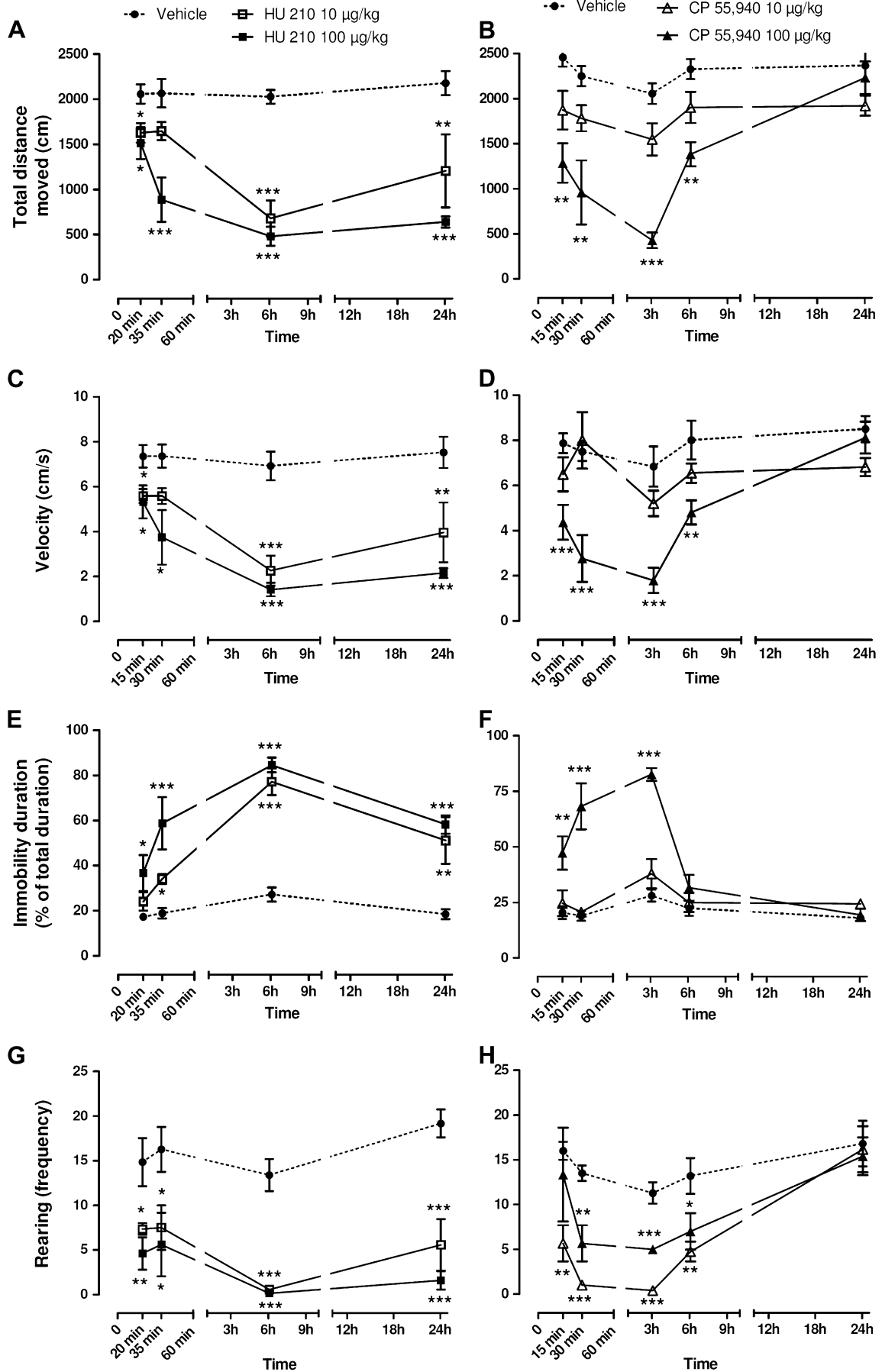
cannabinoid receptors. As a consequence, a combination of four typical behavioural tests (the tetrad) including antinociception, hypomobility, hypothermia and catalepsy has been conventionally used as a screening procedure predictive of cannabinoid identity (Martin et al., 1987, 1991). Furthermore, a correlation was evidenced between the affinity of ligands at the CB₁ cannabinoid receptor and their potency in the tetrad test in rodents (Compton et al., 1993). Hence, in addition to Δ⁹-tetrahydrocannabinol (Δ⁹-THC), recognised as the main psychoactive constituent of the plant *Cannabis sativa* L., several synthetic derivatives authenticated in the tetrad test are now validated as reference ligands for cannabinoid receptors (Ryan et al., 1995; Compton et al., 1996; Wiley et al., 1998; Adams et al., 1995). Notwithstanding the effectiveness of these tests, it is noteworthy that behavioural responses are generally measured within 1 h after administration, even though most cannabinoid ligands are lipophilic and display remarkably long elimination half-life (Grotenhermen, 2003; Harvey and Agurell, 1999).

Given the growing interest in the development of cannabinoids as medicine, a lot of efforts have been concentrated on the design of selective cannabinoid drugs. Despite these intense research most of the current cannabinoid ligands display a lack of specificity for

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cannabinoid receptors (For review see Brown, 2007; Oz, 2006). Besides, though the majority of studies have been focused on cannabinoid mediated inhibition of adenylyl cyclase, it has become clear that several cannabinoid ligands may induce complex cellular signalling pathways (Bosier et al., 2008a,b). These likely reflect the ability of these ligands to promote different conformations of the CB₁ cannabinoid receptor (Anavi-Goffer et al., 2007; Georgieva et al., 2008) resulting in distinct combinations of G protein couplings and activation (Bonhaus et al., 1998; Glass and Northup, 1999; Mukhopadhyay and Howlett, 2005; Lauckner et al., 2005). The concept that unique patterns of G protein activation exist for distinct agonists allows for the expectation that cannabinoid ligands could ultimately produce several related and unrelated physiological and pharmacological responses. Indeed, we previously reported a functional selectivity among the responses associated with the activation of CB₁ cannabinoid receptor by HU 210 and CP 55,940, two commonly used cannabinoid agonists (Bosier et al., 2007, 2009).

Therefore, the present study aimed at further evaluating and comparing the immediate and delayed activities mediated by either HU 210 or CP 55,940, *in vivo*. Spontaneous motor activities and induced catalepsy were monitored to re-examine the behavioural effects mediated by these drugs. As expected for cannabinoid agonists, the exposure to either HU 210 or CP 55,940 produced a rapid reduction of locomotor activity that was reversed by co-administration with the CB₁ selective receptor inverse agonist/antagonist SR 141716A. However HU 210, but not CP 55,940, was found to induce persistent responses, lasting for at least 24 h, which were not inhibited by SR 141716A. Together, these results further shed light on pharmacokinetic as well as pharmacodynamic differences between these reference cannabinoid agonists.

2. Experimental procedures

2.1. Animals

Male Wistar rats obtained from Charles River Laboratories (distributed by Iffa-Credo, Lyon, France) and weighing 275–300 g at the beginning of the experiment were acclimatised in the house facility from the Vrije Universiteit Brussel in a controlled environment (10/14-h dark/light cycle, temperature controlled room) during 1 week. Animals had *ad libitum* access to food and water. All experiments were approved by the local ethical committee and housing conditions were as specified by the Belgian law of 14 November 1993 on the protection of laboratory animals (LA 1230314).

2.2. Drugs

HU 210 and CP 55,940 were purchased from Tocris Cookson (Bristol, UK). The CB₁ cannabinoid receptor inverse agonist/antagonist SR 141716A was generously given by Dr Barth, Sanofi-Synthelabo Research (Montpellier, France). HU 210 and CP 55,940 were prepared as stock solutions in ethanol at 20 mg/ml, stored as aliquots at –80 °C and administered *i.p.* (1 ml/kg) in a 1% ethanol, 1% Tween 80 saline solution. SR 141716A was prepared in 4% ethanol, 1% Tween 80 saline solution, and administered *i.p.* in a volume of 1 ml/kg.

2.3. Catalepsy

At the given time points after the agonist injections, rats were tested for catalepsy by the placement of both forelimbs over a thin metal bar fixed at 10 cm above the ground. Animals were timed for the latency to move one or both forelimbs. Three trials were taken for each animal and the longest latency time on the bar was recorded. Results were expressed in latency seconds on the bar.

2.4. Locomotion

The motor activities of the animals were recorded in an open field device (60 × 60 cm) equipped with a digital video tracking system. Animals were placed in the arena 5 min after the beginning of latency measurement for catalepsy. The session began by placing the rat in the centre of the arena and lasted for 5 min. Behavioural measures recorded were i) the total distance moved, ii) the velocity (during the moving duration), iii) the duration of immobility and iv) rearing (vertical exploratory behaviour). All these measurements were scored with the Noldus EthoVision video tracking system (Wageningen, the Netherlands).

2.5. Procedure

In tests involving only agonists, rats were given a single *i.p.* injection prior monitoring catalepsy and locomotion. The motor effects of HU 210 and CP 55,940 were measured at different time intervals after the injection from 15 min to 24 h. Worth mentioning, preliminary experiments revealed that repeating the behavioural tests on a same animal led to some contextual habituation that altered the reproducibility. Therefore, separate cohorts of animals were systematically used for the measures at different time points. For tests combining agonists and the antagonist SR 141716A, rats were given two separate *i.p.* injections. Different groups of rats received SR 141716A plus the agonist (HU 210 or CP 55,940), vehicle plus the agonist, SR 141716A plus vehicle and vehicle plus vehicle. The doses of HU 210 or CP 55,940 were 10 or 100 µg/kg. SR 141716A was injected at 1 mg/kg or 10 mg/kg.

2.6. Statistical analyses

Data are expressed as mean values with SEM and statistical analyses were performed in comparison to the control animal for each time point by one-way ANOVA followed by Tukey's post-hoc test using the software GraphPad PRISM (San Diego, CA, USA).

3. Results

3.1. Reduced locomotion and catalepsy induced by HU 210 and CP 55,940

As expected for cannabinoid agonists, both HU 210 and CP 55,940 reduced spontaneous locomotion after *i.p.* injections (Fig. 1). Both a reduction in velocity and an increase in the duration of immobility episodes accounted for the reduction in the walking distances, suggesting that the cannabinoid agonists inhibited different components of motor activities. In addition, the frequency of rearing was significantly decreased with both agonists, indicative of a reduction in the vertical exploratory activity. Consistent with previous reports, significant alterations in motor activities by HU 210 and CP 55,940 were evidenced as soon as 20 min after systemic administration (Little et al., 1989; Compton et al., 1991; Martin-Calderon et al., 1998; Mauler et al., 2002). However, analyses of animal behaviour at late time points revealed that the effects induced by HU 210 were maximal 6 h after the administration and persisted for at least 24 h, whereas the maximal responses induced by CP 55,940 occurred 3 h after the injection and were totally reversed after 24 h. Worth noting, the amplitude of the effects induced by HU 210 and CP 55,940 were similar when tested at 100 µg/kg, while at lower dose (10 µg/kg) only HU 210 provoked a significant alteration of the different motor parameters, except for the rearing behaviour.

The decreased spontaneous activity induced by both compounds shortly after the administration was correlated with a typical cataleptic response. However, at variance with the spontaneous locomotor behaviour, the catalepsy induced by HU 210 at the dose of 100 µg/kg was substantially more pronounced than with the lower dose tested (10 µg/kg), suggesting that the potency

Fig. 1. Time-course dependent alteration of spontaneous motor activity induced by HU 210 and CP 55,940. Total distance moved (A, B), average velocity (C, D), duration of immobility episodes (E, F) and rearing frequency (G, H) were measured in different groups of male Wistar rats ($n = 6-12$). HU 210 (10 and 100 µg/kg) (left) or CP 55,940 (10 and 100 µg/kg) (right) was administered 15 min to 24 h prior to the behavioural session. As this session started by the 5 min catalepsy measurement, the different locomotor parameters were measured during a 5 min period exactly 20 min, 35 min, 6 h05 and 24 h05 after the injections. Data points represent the mean values ± SEM. *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$, relative to vehicle injected rats (controls) tested at the same time points (one-way ANOVA followed by Tukey's post-hoc test).

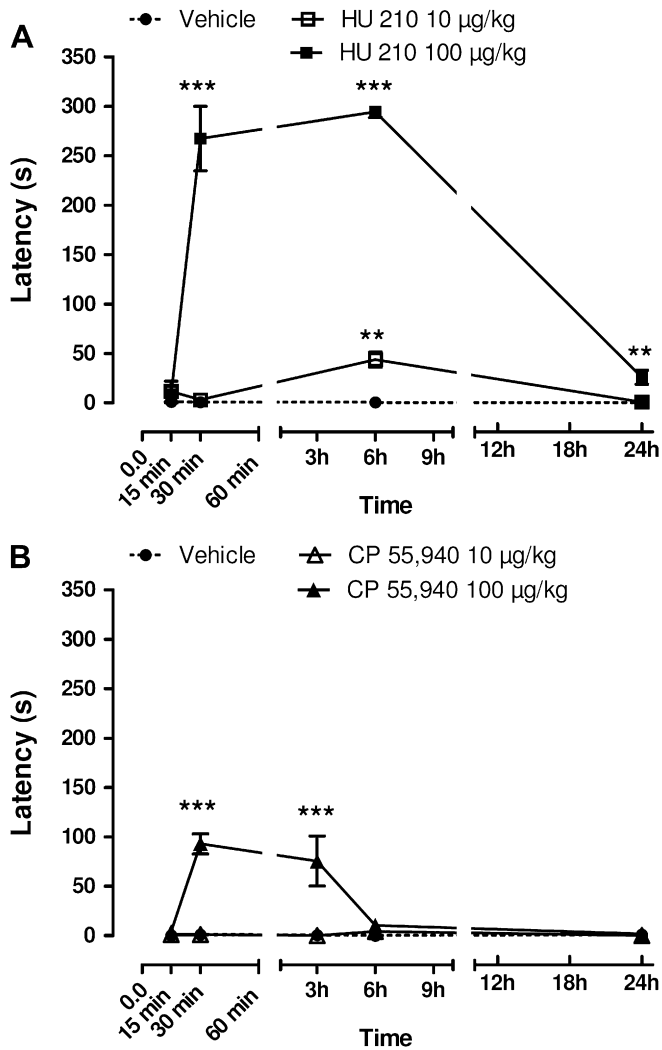


Fig. 2. Time-course dependent alteration of catalepsy-like behaviour induced by HU 210 and CP 55,940. Catalepsy was measured in male Wistar rats ($n = 6-12$) 15 min to 24 h consecutively to HU 210 (10 and 100 $\mu\text{g}/\text{kg}$) (A) or CP 55,940 (10 and 100 $\mu\text{g}/\text{kg}$) (B) injections. The different time points were 15 min, 30 min, 6 h or 24 h. The latency to remove the forelimbs from the fixed bar was used to quantify the catalepsy. Results shown are mean latency values \pm SEM. *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$, relative to vehicle injected rats (controls) tested at the same time points (one-way ANOVA followed by Tukey's post-hoc test).

of this agonist was different in these two assays (Fig. 2A). In addition, prolonged monitoring of catalepsy after a single administration revealed that the effects of HU 210 were no longer evident after 24 h. Likewise, the cataleptic behaviour induced by CP 55,940 was of shorter duration as compared to the hypolocomotion (Fig. 2B).

3.2. Effect of SR 141716A on the early cannabinoid-induced hypolocomotion and catalepsy

The general assumption that the CB₁ cannabinoid receptor accounts for the motor behavioural responses to cannabinoids was further assessed by co-administration of the CB₁ selective inverse agonist/antagonist SR 141716A. Intraperitoneal administration of 1 mg/kg SR 141716A did not alter ambulation, rearing or catalepsy (Fig. 3). However, as expected, administration of this antagonist nearly totally inhibited HU 210- and CP 55,940-induced catalepsy and hypolocomotion when examined 30 and 35 min post-injection (Fig. 3). Nevertheless, at the same dose, SR 141716A failed to restore the rearing behaviour.

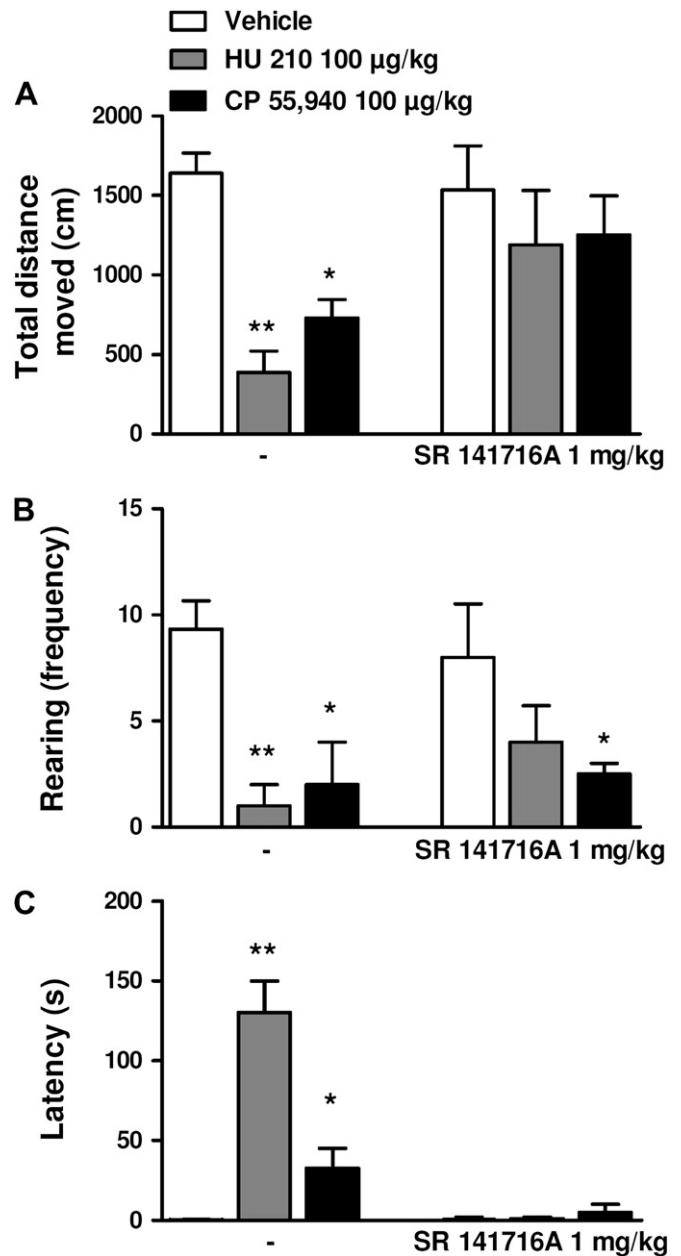


Fig. 3. Influence of SR 141716A on the short-term mediated changes in motor activities induced by cannabinoids. The effects of HU 210 (100 $\mu\text{g}/\text{kg}$) or CP 55,940 (100 $\mu\text{g}/\text{kg}$) were measured in combination with SR 141716A (1 mg/kg) on locomotion (total distance moved) (A), rearing (frequency) (B) and catalepsy (latency) (C) in different groups of male Wistar rats (at least $n = 3$). SR 141716A was injected 40 min prior to the session onset. HU 210 and CP 55,940 were administered 30 min or 35 min before catalepsy and locomotion assessments, respectively. Data points represent the mean values \pm SEM. ** $p < 0.01$, * $p < 0.05$, relative to vehicle injected rats (controls) tested at the same time points (one-way ANOVA followed by Tukey's post-hoc test).

3.3. Effect of SR 141716A on the long-lasting cannabinoid-induced hypolocomotion and catalepsy

Considering the sustained responses observed with HU 210 on the spontaneous locomotor activity, the influence of selectively inhibiting the CB₁ cannabinoid receptor was further characterized at late time points after administration of the agonists. Thus, SR 141716A (1 mg/kg) was administered 10 min before a single injection of HU 210 or CP 55,940 and the behavioural assessments were conducted

6 h or 3 h later, respectively. At variance with the inhibition validated for early responses, SR 141716A failed to reverse the decline of locomotion as well as the reduction of rearing induced 6 h after the administration of HU 210 (Fig. 4A and C) even when the agonist was tested at the lower dose of 10 µg/kg. Similarly, the hypolocomotion observed 24 h after the administration of HU 210 was not reversed by SR 141716A (data not shown). Confirming the putative differences in the responses to HU 210 and CP 55,940, increasing the concentration of SR 141716A to 10 mg/kg failed to reverse the alterations of motor activities mediated by HU 210, whereas 1 mg/kg was sufficient to consistently suppress both the reduction of locomotion or the decreased rearing mediated by 100 µg/kg CP 55,940 (Fig. 4B and D). Of note, SR 141716A *per se* caused a significant increase in horizontal ambulation when administered at 10 mg/kg *i.p.*

Nevertheless, when focusing on the cataleptic behaviour, a single administration of SR 141716A was proven effective in antagonizing both early and late cannabinoid agonist-mediated responses. Thus, SR 141716A (1 or 10 mg/kg) completely abolished the catalepsy induced by HU 210 when examined 6 h after the injection (Fig. 4E). Likewise, the maximal response to CP 55,940 measured 3 h after administration was nearly totally abrogated by the co-administration of SR 141716A (Fig. 4F).

Finally, in order to exclude the involvement of late cannabinoid receptor activation in the sustained HU 210-mediated locomotor

effects, we designed an additional experiment in which HU 210 was first administered to the rat (100 µg/kg, 6 h before behavioural assessment) and SR 141716A was administered, 40 min only before catalepsy and locomotion measurements. Indeed, this timing for SR 141716A administration was proven effective in the short-term experiments (Fig. 3). In these conditions, the antagonist was also found ineffective in blocking the late locomotor and rearing behaviours responses induced by HU 210 (Fig. 5A and B). Nevertheless, when used in these conditions, the antagonist was ineffective in preventing the long-lasting HU 210-induced catalepsy while it efficiently blocked the agonist-induced catalepsy when administered before HU 210 (Fig. 5C).

4. Discussion

Consistent with the expression of CB₁ cannabinoid receptors in motor-related brain structures such as the cerebellum and the basal ganglia, altered locomotor behaviour is commonly observed after exposure of rodents to cannabinoid ligands. Accordingly, minutes after administration in rats, HU 210 and CP 55,940 were herein found to reduce spontaneous locomotor activities and provoke catalepsy-like behaviour through activation of the CB₁ cannabinoid receptor. However an original finding of the present study is that in addition to these rapidly evoked responses, HU 210 also induced

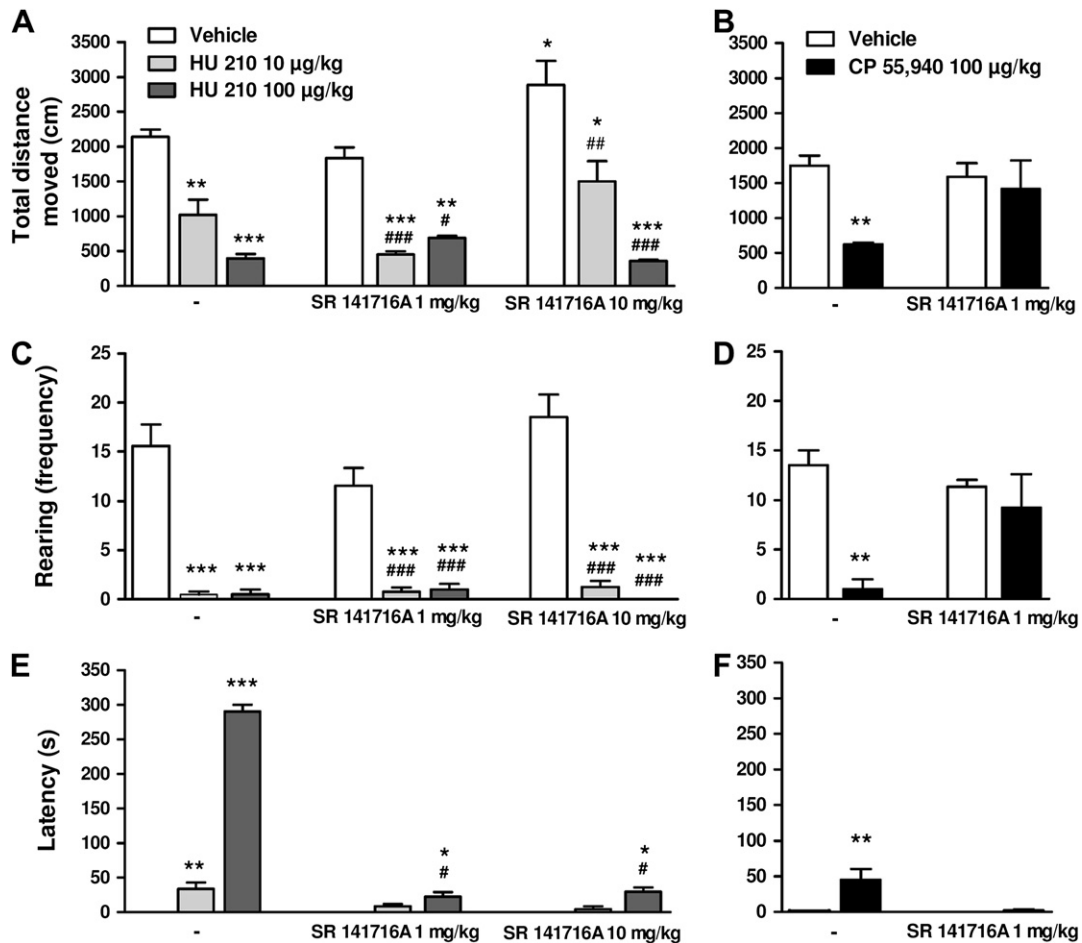


Fig. 4. Influence of SR 141716A on the sustained changes in motor activities induced by cannabinoids. Locomotion (total distance moved) (A, B), rearing (frequency) (C, D) and catalepsy (latency) (E, F) were measured in different groups of male Wistar rats (n = 4–6). HU 210 (10 and 100 µg/kg) (left) or CP 55,940 (10 and 100 µg/kg) (right) was injected in combination with SR 141716A (1 and 10 mg/kg), 6 h and 3 h prior to behavioural assessments, respectively. SR 141716A was injected 10 min before agonist administration. Data points represent the mean values ± SEM. One-Way ANOVA followed by Tukey's post-hoc test ***p < 0.001, **p < 0.01, *p < 0.05, relative to vehicle injected rats (controls); ###p < 0.001, ##p < 0.01, #p < 0.05, relative to rats treated with indicated doses of SR 141716A.

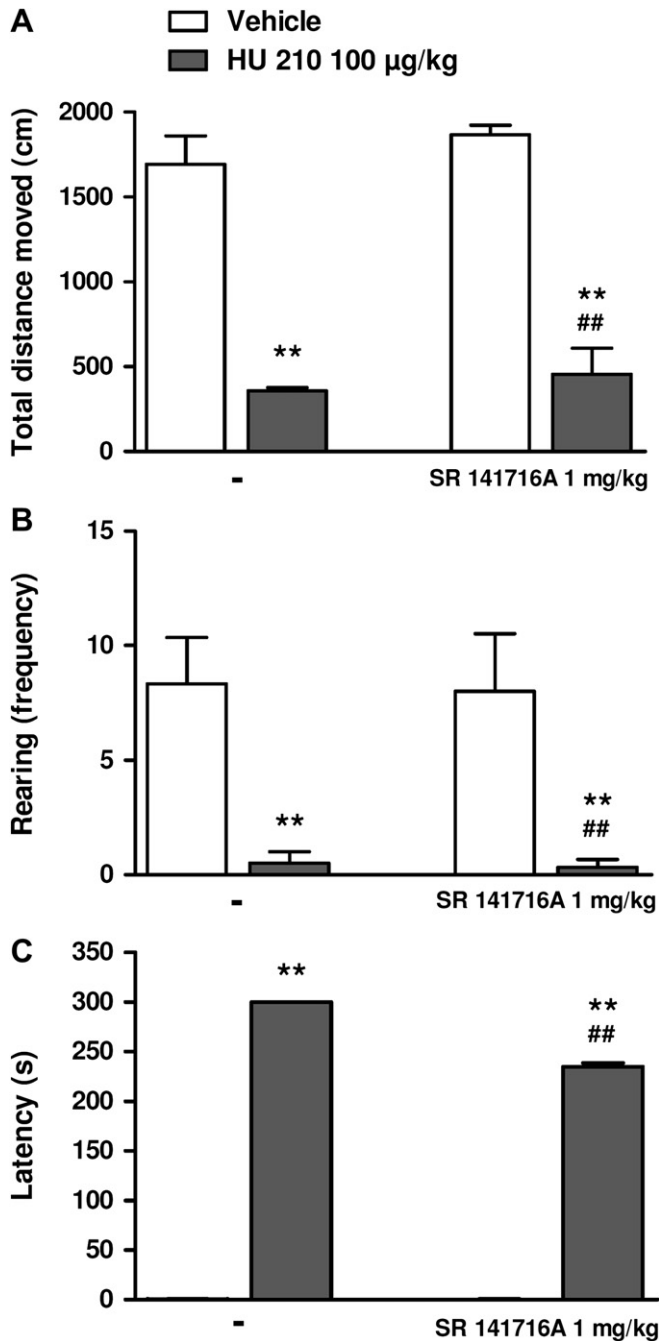


Fig. 5. Influence of delayed administration of SR 141716A on the persistent HU 210-mediated changes in locomotor activities. Locomotion (total distance moved) (A), rearing (frequency) (B) and catalepsy (latency) (C) were measured in different groups of male Wistar rats ($n = 4$). HU 210 100 µg/kg was injected 6 h prior to behavioural assessments while SR 141716A (1 mg/kg) or the corresponding vehicle was injected 40 min before behavioural experiments. Data points represent the mean values with SEM. One-way ANOVA followed by Tukey's post-hoc test, ** $p < 0.01$, * $p < 0.05$, relative to vehicle/vehicle injected rats (controls), ## $p < 0.01$, # $p < 0.05$, relative to rats treated with SR 141716A alone.

sustained hypolocomotion which was not reversed by the CB₁ selective inverse agonist/antagonist SR 141716A. This suggests that all motor behaviours caused by HU 210 are not mediated through CB₁ cannabinoid receptors, a hypothesis that is also supported by time-course studies.

Typically, in early reports evidencing the central effects mediated by cannabinoid agonists, the behavioural responses were

systematically examined 5–60 min after drug administration (Martin et al., 1987; Little et al., 1989; Adams et al., 1995; Ryan et al., 1995; Wiley et al., 1998). More recently, other studies conducted on longer periods of time, up to 24 h, have indicated that the behavioural changes triggered by HU 210 and CP 55,940 as well as Δ^9 -THC and WIN 55212-2 were maximal from 1 to 2 h after a single i.p. injection and were not longer detected after 4 h (Martin-Calderon et al., 1998; Mauler et al., 2002; McMahon and Koek, 2007). Supporting such short duration of action, ex vivo binding studies have revealed maximal CB₁ cannabinoid receptor occupancy in the brain 30 min after i.p. injection and compounds tested were eliminated from the brain within 4 h after administration (Petitet et al., 1999). While we herein observed that both HU 210 and CP 55,940 rapidly altered behaviours after administration, we also evidenced that the response to HU 210 is much more prolonged as compared to CP 55,940. Furthermore, the motor effects of HU 210 are much more sustained than previously reported, persisting for at least 24 h after a single i.p. injection. At variance with previous reports showing that the catalepsy induced by Δ^9 -THC and WIN 55,212-2 lasts longer than hypolocomotion and other behavioural responses (McMahon and Koek, 2007), we observed that catalepsy induced by HU 210 or CP 55,940 develops later, and lasts shorter than hypolocomotion. Besides, when HU 210 was injected first and SR 141716A secondly, respectively 6 h and 40 min before the measurement of catalepsy, the antagonist failed to prevent catalepsy. This suggests that an initial stimulation of CB₁ cannabinoid receptor is sufficient to trigger persistent behavioural responses.

In addition to these differences in the time-course of the responses mediated by the agonists, dissimilarities between HU 210 and CP 55,940 were also evidenced when considering their intrinsic pharmacological profiles. Indeed, it was previously suggested that affinity (Compton et al., 1993) and/or efficacy and potency (Burkey et al., 1997) values are key parameters connecting receptor occupancy to drug-mediated functional responses *in vivo*. As comparable efficacy was determined for HU 210 and CP 55,940 in functional [³⁵S]-GTP γ S binding assays in rat brain membranes (Govaerts et al., 2004), a similar dose-response relationship would be expected for these drugs in altering motor behaviours. While the dose-dependent profile was evident for CP 55,940-induced hypolocomotion and catalepsy, this was not observed for HU 210. Thus, at doses producing dose-dependent catalepsy, HU 210 was found to elicit a maximal effect on locomotion 6 h after injection. In addition to some previously reported partial agonist properties of HU 210 (Sugiura et al., 1999; Martin et al., 2000), these inconsistencies in the dose-dependent profile of HU 210-mediated responses, suggest the existence of additional mechanisms than the simple CB₁ cannabinoid receptor-mediated-on/off G protein activation (for review also see Howlett et al., 2002).

The failure of SR 141716A in blocking the locomotor activities modulated by certain cannabinoid ligands was previously reported (Jarbe et al., 2003; McMahon and Koek, 2007). As SR 141716A *per se* was also shown to induce hypoactivity (Jarbe et al., 2002, 2006; McMahon and Koek, 2007), it was suggested that the lack or incomplete antagonism might be due to direct effects provoked by this antagonist (McMahon and Koek, 2007). While the present study also evidenced some limitation in the blocking properties of SR 141716A on HU 210-induced hypolocomotion, our results are not consistent with this hypothesis. Indeed, rather than reducing locomotion, SR 141716A caused a significant increase in locomotion when tested at maximal dosages. Similar observations have already been reported (Costa and Colleoni, 1999; Compton et al., 1996; Cosenza et al., 2000; Bass et al., 2002) and are in better agreement with the inverse agonism profile of SR 141716A (Bouaboula et al., 1997). Together with these previous findings, our observations more likely suggest that all behavioural changes induced by

HU 210 do not result from the activation of the CB₁ cannabinoid receptor.

An apparent incomplete functional antagonism of HU 210 by SR 141716A could possibly result from distinct pharmacokinetic properties or from a rapid metabolism of the latter. However, data from human studies show that in a typical dose range, the elimination half-life of SR 141716A is between 6 and 9 days (Padwal and Majumdar, 2007). In addition, in rats, 50% of the brain cannabinoid receptors remain occupied 8 h after oral administration of SR 141716A (10 mg/kg), supporting that this antagonist has a long duration of action into the brain. According with this, efficient antagonism of behavioural responses to cannabinoids was previously reported up to 24 h after administration of SR 141716A (Compton et al., 1996). Also, we herein observed that 6 h and even 24 h (not shown) after administration, SR 141716A totally prevented the catalepsy induced by HU 210. Finally, when SR 141716A was injected 40 min before behavioural testing of rats treated 6 h earlier with HU 210, the locomotor activity was not restored to any further extent. As we show the effectiveness of this 40 min treatment with the antagonist in the prevention of the early effect of HU 210, this further demonstrates the lack of CB₁ cannabinoid receptor involvement in the sustained HU 210 effect, even through a late CB₁ cannabinoid receptor activation. Together, these data imply that SR 141716A remains distributed throughout the brain and is able to prevent the CB₁ cannabinoid receptor-mediated responses during at least 24 h.

Several CB₁ cannabinoid receptor-independent responses have already been demonstrated *in vivo* with both endogenous and exogenous cannabinoid ligands including anandamide (Smith et al., 1998; Di Marzo et al., 2000), methanandamide (Jarbe et al., 2003; Baskfield et al., 2004), WIN 55,212-2 (Pistis et al., 2004), CP 55,940 (Hajos et al., 2001) and Δ^9 -THC (O'Sullivan et al., 2005). However, the present study constitutes one of the first report demonstrating persistent *in vivo* effects of HU 210 that are not reversed by SR 141716A. The interaction of HU 210 on another cannabinoid receptor could indeed explain the functional differences between HU 210 and CP 55,940. Corroborating the existence of additional binding sites for this drug, HU 210 was recently described as a potent agonist of the novel cannabinoid receptor GPR55 (Ryberg et al., 2007). In addition HU 210 was shown to display positive allosteric modulators properties at glycine receptors (Demir et al., 2009). Nevertheless, in other investigations, HU 210 failed to activate GPR55 (Oka et al., 2007). In addition some studies reported a failure of HU 210 to activate G proteins through CB₁ receptor-independent mechanism whereas both AEA and WIN 55,212-2 were shown to induce G protein activation through mechanisms not directly related to the CB₁ cannabinoid receptor. While the existence of unspecific targets was already speculated from some *in vitro* reports (Lau and Chow, 2003; Rao and Kaminski, 2006; Gustafsson et al., 2009; Fowler et al., 2010) this study provides a direct evidence for unspecific action of HU 210 in behavioural paradigms classically used to characterise cannabinoid ligands.

Together this study shows that a single injection of HU 210 provokes persistent effects on locomotion up to 24 h consecutively to the administration. At variance, the effects of CP 55,940 were not longer evident at this time, revealing considerable differences in the response kinetic to these two ligands when tested *in vivo*. Furthermore, it appears that although HU 210 and CP 55,940 share common properties, their combination with a CB₁ cannabinoid receptor inverse agonist reveals complex differences in their pharmacodynamic profiles. Indeed, the data presented in this study are consistent with the involvement of an additional target in some sustained responses to HU 210. As several *in vivo* studies take advantage of HU 210 as a high affinity and high efficacy reference agonist to evaluate the CB₁ cannabinoid receptor related functions, it is important to consider the existence some off-target effects when interpreting these data.

References

- Adams, I.B., Ryan, W., Singer, M., Thomas, B.F., Compton, D.R., Razdan, R.K., Martin, B.R., 1995. Evaluation of cannabinoid receptor binding and *in vivo* activities for anandamide analogs. *J. Pharmacol. Exp. Ther.* 273, 1172–1181.
- Anavi-Goffer, S., Fleischer, D., Hurst, D.P., Lynch, D.L., Barnett-Norris, J., Shi, S., Lewis, D.L., Mukhopadhyay, S., Howlett, A.C., Reggio, P.H., Abood, M.E., 2007. Helix 8 Leu in the CB₁ cannabinoid receptor contributes to selective signal transduction mechanisms. *J. Biol. Chem.* 282, 25100–25113.
- Baskfield, C.Y., Martin, B.R., Wiley, J.L., 2004. Differential effects of delta9-tetrahydrocannabinol and methanandamide in CB₁ knockout and wild-type mice. *J. Pharmacol. Exp. Ther.* 309, 86–91.
- Bass, C.E., Griffin, G., Grier, M., Mahadevan, A., Razdan, R.K., Martin, B.R., 2002. SR-141716A-induced stimulation of locomotor activity. A structure–activity relationship study. *Pharmacol. Biochem. Behav.* 74, 31–40.
- Bonhaus, D.W., Chang, L.K., Kwan, J., Martin, G.R., 1998. Dual activation and inhibition of adenylyl cyclase by cannabinoid receptor agonists: evidence for agonist-specific trafficking of intracellular responses. *J. Pharmacol. Exp. Ther.* 287, 884–888.
- Bosier, B., Hermans, E., Lambert, D., 2008a. Differential modulation of AP-1- and CRE-driven transcription by cannabinoid agonists emphasizes functional selectivity at the CB₁ receptor. *Br. J. Pharmacol.* 155, 24–33.
- Bosier, B., Hermans, E., Lambert, D.M., 2009. Concomitant activation of adenylyl cyclase suppresses the opposite influences of CB₁ cannabinoid receptor agonists on tyrosine hydroxylase expression. *Biochem. Pharmacol.* 77, 216–227.
- Bosier, B., Lambert, D.M., Hermans, E., 2008b. Reciprocal influences of CB₁ cannabinoid receptor agonists on ERK and JNK signalling in N1E-115 cells. *FEBS Lett.* 582, 3861–3867.
- Bosier, B., Tilleux, S., Najimi, M., Lambert, D.M., Hermans, E., 2007. Agonist selective modulation of tyrosine hydroxylase expression by cannabinoid ligands in a murine neuroblastoma cell line. *J. Neurochem.* 102, 1996–2007.
- Bouaboula, M., Perrachon, S., Milligan, L., Canat, X., Rinaldi-Carmona, M., Portier, M., Barth, F., Calandra, B., Pecceu, F., Lupker, J., Maffrand, J.P., Le Fur, G., Casellas, P., 1997. A selective inverse agonist for central cannabinoid receptor inhibits mitogen-activated protein kinase activation stimulated by insulin or insulin-like growth factor 1. Evidence for a new model of receptor/ligand interactions. *J. Biol. Chem.* 272, 22330–22339.
- Brown, A.J., 2007. Novel cannabinoid receptors. *Br. J. Pharmacol.* 152, 567–575.
- Burkey, T.H., Quock, R.M., Consroe, P., Ehler, F.J., Hosohata, Y., Roeske, W.R., Yamamura, H.I., 1997. Relative efficacies of cannabinoid CB₁ receptor agonists in the mouse brain. *Eur. J. Pharmacol.* 336, 295–298.
- Compton, D.R., Aceto, M.D., Lowe, J., Martin, B.R., 1996. *In vivo* characterization of a specific cannabinoid receptor antagonist (SR141716A): inhibition of delta 9-tetrahydrocannabinol-induced responses and apparent agonist activity. *J. Pharmacol. Exp. Ther.* 277, 586–594.
- Compton, D.R., Prescott Jr., W.R., Martin, B.R., Siegel, C., Gordon, P.M., Razdan, R.K., 1991. Synthesis and pharmacological evaluation of ether and related analogues of delta 8-, delta 9-, and delta 9,11-tetrahydrocannabinol. *J. Med. Chem.* 34, 3310–3316.
- Compton, D.R., Rice, K.C., De Costa, B.R., Razdan, R.K., Melvin, L.S., Johnson, M.R., Martin, B.R., 1993. Cannabinoid structure–activity relationships: correlation of receptor binding and *in vivo* activities. *J. Pharmacol. Exp. Ther.* 265, 218–226.
- Cosenza, M., Gifford, A.N., Gatley, S.J., Pyatt, B., Liu, Q., Makriyannis, A., Volkow, N.D., 2000. Locomotor activity and occupancy of brain cannabinoid CB₁ receptors by the antagonist/inverse agonist AM281. *Synapse* 38, 477–482.
- Costa, B., Colleoni, M., 1999. SR141716A induces in rats a behavioral pattern opposite to that of CB₁ receptor agonists. *Zhongguo Yao Li Xue. Bao.* 20, 1103–1108.
- Demir, R., Leuwer, M., de la Rochea, J., Krampfl, K., Foadi, N., Karst, M., Dengler, R., Haeseler, G., Ahrens, J., 2009. Modulation of glycine receptor function by the synthetic cannabinoid HU210. *Pharmacology* 83, 270–274.
- Di Marzo, V., Breivogel, C.S., Tao, Q., Bridgen, D.T., Razdan, R.K., Zimmer, A.M., Zimmer, A., Martin, B.R., 2000. Levels, metabolism, and pharmacological activity of anandamide in CB₁ cannabinoid receptor knockout mice: evidence for non-CB₁, non-CB₂ receptor-mediated actions of anandamide in mouse brain. *J. Neurochem.* 75, 2434–2444.
- Fowler, C.J., Gustafsson, S.B., Chung, S.C., Persson, E., Jacobsson, S.O., Bergh, A., 2010. Targeting the endocannabinoid system for the treatment of cancer – a practical view. *Curr. Top. Med. Chem.* 10, 814–827.
- Georgieva, T., Devanathan, S., Stropova, D., Park, C.K., Salamon, Z., Tollin, G., Hruby, V.J., Roeske, W.R., Yamamura, H.I., Varga, E., 2008. Unique agonist-bound cannabinoid CB₁ receptor conformations indicate agonist specificity in signaling. *Eur. J. Pharmacol.* 581, 19–29.
- Glass, M., Northup, J.K., 1999. Agonist selective regulation of G proteins by cannabinoid CB₁(1) and CB₂(2) receptors. *Mol. Pharmacol.* 56, 1362–1369.
- Govaerts, S.J., Hermans, E., Lambert, D.M., 2004. Comparison of cannabinoid ligands affinities and efficacies in murine tissues and in transfected cells expressing human recombinant cannabinoid receptors. *Eur. J. Pharm. Sci.* 23, 233–243.
- Grotenhermen, F., 2003. Pharmacokinetics and pharmacodynamics of cannabinoids. *Clin. Pharmacokinet.* 42, 327–360.
- Gustafsson, S.B., Lindgren, T., Jonsson, M., Jacobsson, S.O., 2009. Cannabinoid receptor-independent cytotoxic effects of cannabinoids in human colorectal carcinoma cells: synergism with 5-fluorouracil. *Cancer Chemother. Pharmacol.* 63, 691–701.
- Hajos, N., Ledent, C., Freund, T.F., 2001. Novel cannabinoid-sensitive receptor mediates inhibition of glutamatergic synaptic transmission in the hippocampus. *Neuroscience* 106, 1–4.

- Harvey, D.J., Agurell, S., 1999. Absorption, distribution, and biotransformation of the cannabinoids. *Marijuana and Medicine*. Humana Press Inc, Totowa, New Jersey, pp. 91–103.
- Howlett, A.C., Barth, F., Bonner, T.I., Cabral, G., Casellas, P., Devane, W.A., Felder, C.C., Herkenham, M., Mackie, K., Martin, B.R., Mechoulam, R., Pertwee, R.G., 2002. International Union of Pharmacology. XXVII. Classification of cannabinoid receptors. *Pharmacol. Rev.* 54, 161–202.
- Jarbe, T.U., Andrzejewski, M.E., DiPatrizio, N.V., 2002. Interactions between the CB1 receptor agonist delta 9-THC and the CB1 receptor antagonist SR-141716 in rats: open-field revisited. *Pharmacol. Biochem. Behav.* 73, 911–919.
- Jarbe, T.U., DiPatrizio, N.V., Li, C., Makriyannis, A., 2003. The cannabinoid receptor antagonist SR-141716 does not readily antagonize open-field effects induced by the cannabinoid receptor agonist (R)-methanandamide in rats. *Pharmacol. Biochem. Behav.* 75, 809–821.
- Jarbe, T.U., Ross, T., DiPatrizio, N.V., Pandarinathan, L., Makriyannis, A., 2006. Effects of the CB1R agonist WIN-55,212-2 and the CB1R antagonists SR-141716 and AM-1387: open-field examination in rats. *Pharmacol. Biochem. Behav.* 85, 243–252.
- Lau, A.H., Chow, S.S., 2003. Effects of cannabinoid receptor agonists on immunologically induced histamine release from rat peritoneal mast cells. *Eur. J. Pharmacol.* 464, 229–235.
- Lauckner, J.E., Hille, B., Mackie, K., 2005. The cannabinoid agonist WIN55,212-2 increases intracellular calcium via CB1 receptor coupling to Gq/11 G proteins. *Proc. Natl. Acad. Sci. U.S.A.* 102, 19144–19149.
- Little, P.J., Compton, D.R., Mechoulam, R., Martin, B.R., 1989. Stereochemical effects of 11-OH-delta 8-THC-dimethylheptyl in mice and dogs. *Pharmacol. Biochem. Behav.* 32, 661–666.
- Martin, B.R., Compton, D.R., Little, P.J., Martin, T.J., Beardsley, P.M., 1987. Pharmacological evaluation of agonistic and antagonistic activity of cannabinoids. *NIDA Res. Monogr.* 79, 108–122.
- Martin, B.R., Compton, D.R., Thomas, B.F., Prescott, W.R., Little, P.J., Razdan, R.K., Johnson, M.R., Melvin, L.S., Mechoulam, R., Ward, S.J., 1991. Behavioral, biochemical, and molecular modeling evaluations of cannabinoid analogs. *Pharmacol. Biochem. Behav.* 40, 471–478.
- Martin, R.S., Luong, L.A., Welsh, N.J., Eglon, R.M., Martin, G.R., MacLennan, S.J., 2000. Effects of cannabinoid receptor agonists on neuronally-evoked contractions of urinary bladder tissues isolated from rat, mouse, pig, dog, monkey and human. *Br. J. Pharmacol.* 129, 1707–1715.
- Martin-Calderon, J.L., Munoz, R.M., Villanua, M.A., del Arco, I., Moreno, J.L., de Fonseca, F.R., Navarro, M., 1998. Characterization of the acute endocrine actions of (-)-11-hydroxy-delta8-tetrahydrocannabinol-dimethylheptyl (HU-210), a potent synthetic cannabinoid in rats. *Eur. J. Pharmacol.* 344, 77–86.
- Mauler, F., Mittendorf, J., Horvath, E., De Vry, J., 2002. Characterization of the diarylether sulfonylester (-)-(R)-3-(2-hydroxymethylindanyl-4-oxy)phenyl-4,4,4-trifluoro-1-sulfonate (BAY 38-7271) as a potent cannabinoid receptor agonist with neuroprotective properties. *J. Pharmacol. Exp. Ther.* 302, 359–368.
- McMahon, L.R., Koek, W., 2007. Differences in the relative potency of SR 141716A and AM 251 as antagonists of various in vivo effects of cannabinoid agonists in C57BL/6j mice. *Eur. J. Pharmacol.* 569, 70–76.
- Mukhopadhyay, S., Howlett, A.C., 2005. Chemically distinct ligands promote differential CB1 cannabinoid receptor-Gi protein interactions. *Mol. Pharmacol.* 67, 2016–2024.
- O'Sullivan, S.E., Tarling, E.J., Bennett, A.J., Kendall, D.A., Randall, M.D., 2005. Novel time-dependent vascular actions of delta9-tetrahydrocannabinol mediated by peroxisome proliferator-activated receptor gamma. *Biochem. Biophys. Res. Commun.* 337, 824–831.
- Oka, S., Nakajima, K., Yamashita, A., Kishimoto, S., Sugiura, T., 2007. Identification of GPR55 as a lysophosphatidylinositol receptor. *Biochem. Biophys. Res. Commun.* 362, 928–934.
- Oz, M., 2006. Receptor-independent effects of endocannabinoids on ion channels. *Curr. Pharm. Des.* 12, 227–239.
- Pacher, P., Batkai, S., Kunos, G., 2006. The endocannabinoid system as an emerging target of pharmacotherapy. *Pharmacol. Rev.* 58, 389–462.
- Padwal, R.S., Majumdar, S.R., 2007. Drug treatments for obesity: orlistat, sibutramine, and rimonabant. *Lancet* 369, 71–77.
- Petitot, F., Jeantaud, B., Bertrand, P., Imperato, A., 1999. Cannabinoid penetration into mouse brain as determined by ex vivo binding. *Eur. J. Pharmacol.* 374, 417–421.
- Pistis, M., Perra, S., Pillolla, G., Melis, M., Gessa, G.L., Muntoni, A.L., 2004. Cannabinoids modulate neuronal firing in the rat basolateral amygdala: evidence for CB1- and non-CB1-mediated actions. *Neuropharmacology* 46, 115–125.
- Rao, G.K., Kaminski, N.E., 2006. Cannabinoid-mediated elevation of intracellular calcium: a structure–activity relationship. *J. Pharmacol. Exp. Ther.* 317, 820–829.
- Ryan, W., Singer, M., Razdan, R.K., Compton, D.R., Martin, B.R., 1995. A novel class of potent tetrahydrocannabinols (THCS): 2'-yne-delta 8- and delta 9-THCS. *Life Sci.* 56, 2013–2020.
- Ryberg, E., Larsson, N., Sjogren, S., Hjorth, S., Hermansson, N.O., Leonova, J., Elebring, T., Nilsson, K., Drmota, T., Greasley, P.J., 2007. The orphan receptor GPR55 is a novel cannabinoid receptor. *Br. J. Pharmacol.* 152, 1092–1101.
- Smith, F.L., Fujimori, K., Lowe, J., Welch, S.P., 1998. Characterization of delta9-tetrahydrocannabinol and anandamide antinociception in nonarthritic and arthritic rats. *Pharmacol. Biochem. Behav.* 60, 183–191.
- Sugiura, T., Kodaka, T., Nakane, S., Miyashita, T., Kondo, S., Suhara, Y., Takayama, H., Waku, K., Seki, C., Baba, N., Ishima, Y., 1999. Evidence that the cannabinoid CB1 receptor is a 2-arachidonoylglycerol receptor. Structure–activity relationship of 2-arachidonoylglycerol, ether-linked analogues, and related compounds. *J. Biol. Chem.* 274, 2794–2801.
- Wiley, J.L., Compton, D.R., Dai, D., Lainton, J.A., Phillips, M., Huffman, J.W., Martin, B.R., 1998. Structure–activity relationships of indole- and pyrrole-derived cannabinoids. *J. Pharmacol. Exp. Ther.* 285, 995–1004.