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Involvement of other neurotransmitters in behaviors induced by the cannabinoid CB_1 receptor antagonist SR 141716A in naive mice

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Summary. The receptor mechanisms by which the selective cannabinoid CB_1 receptor antagonist/inverse agonist, SR 141716A [N-piperidino-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-3-pyrazole-carboxamide] produces scratching and head-twitch response (HTR) in naive mice were examined. Acute intraperitoneal administration of varying doses of SR 141716A produced both scratchings (ED_{50} = 3.9 mg/kg) and head-twitches (ED_{50} = 4.6 mg/kg) in a dose-dependent manner. A dose of 10 mg/kg SR 141716A was used to induce the cited behaviors for drug interaction studies. The selective 5-$HT_{2A/C}$ receptor antagonist, SR 46349B [trans-4-[(3Z)3-(2-dimethylaminoethyl) oxyimino-3-(2-fluorophenyl) propen-1-yl] phenol] potently and completely blocked the head-twitches produced by SR 141716A (ID_{50} = 0.08 mg/kg). The induced scratching behavior was partially (68%) and less potently (ID_{50} = 0.6 mg/kg) blocked by SR 46349B pretreatment. The AMPA/kainate receptor antagonist, CNQX [6-cyano-7-nitroquinoxaline-2,3-dione), partially attenuated (68–78%) the induced scratching and head-twitching behaviors. On the contrary, the selective NMDA antagonist, AP-3 [(±)-2-amino-3-phosphonopropionic acid), had no significant effect on these behaviors. The selective tachykinin NK_1 antagonist, CP 94, 994 [(±)-(2S, 3S)-3-(2-methoxybenzylamino)-2-phenylpiperidine], also partially attenuated both the scratching (64%) and the head-twitching (76%) symptoms produced by SR 141716A. Since SR 141716A lacks affinity for the discussed receptors, it appears that the induction of the cited behaviors probably involve indirect activation of their respective neurotransmitter systems.

Keywords: SR 141716A, SR 46349B, CNQX, AP-3, CP 94, 994, scratching, head-twitch, serotonin, tachykinin, glutamate.

Introduction

Although serious research on marijuana began in 1960s, this area of investigation has undergone a renaissance in the last five years. Currently, at least two types of cannabinoid receptors are identified which are designated as cannabinoid central CB_1 and peripheral CB_2 (Reviews: Pertwee, 1997; Matsuda, 1997). Delta-9-tetrahydrocannabinol (Δ^9-THC) is the predominant active compound in the marijuana plant and currently a large number of highly potent synthetic cannabinoid agonists are also available (Reviews: Pertwee, 1997; Shohami et al., 1996). To date, the most potent, selective and well characterized cannabinoid CB_1 receptor antagonist is N-piperidino-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-3-pyrazole-carboxamide (SR 141716A) which has little affinity for CB_2 and many other receptors (Rinaldi-Carmona et al., 1994). Several studies have demonstrated that a challenge injection of SR 141716A precipitates robust symptoms of withdrawal syndrome in rats and mice chronically exposed to either Δ^9-THC (Aceto et al., 1995, 1996; Cook et al., 1998; Tsou et al., 1995), or to a synthetic cannabinoid agonist CP 55, 940 (Rubino et al., 1998). However, some but not all of these studies show that SR 141716A by itself produces wet-dog shakes and lateral scratchings in nontolerant chronically vehicle-exposed control animals. The SR 141716A-induced behaviors in nontolerant animals may be a reflection of: 1) its intrinsic activity on the cannabinoid CB_1 receptor since SR 141716A also possesses inverse agonist activity in some cannabinoid functional models (Compton et al., 1996; Landsman et al., 1997); 2) antagonism of action of an endogenous cannabinoid; or 3) its possible direct or indirect action on noncannabinoid neurotransmitter systems. Activation of several neurotransmitter systems may result in production of wet-dog shakes in rats [or its behavioral homologue the head-twitch response (HTR) in mice] as well as lateral scratching behavior in rodents. In fact, excitatory aminoacids (such as kainate or domoic acid) and tachykinin receptor agonists (such as substance P or neurokinin A) induce the cited behaviors in rodents (Itoi et al., 1992; Mjellem et al., 1993; Olney et al., 1974; Sakurada et al., 1991; Stoessl et al., 1987; Tasker et al., 1991; Velazquez et al., 1997; Worms et al., 1981). Many of the latter studies suggest that these agents seem to act upstream to the serotonergic system in initiating the cited behaviors. Indeed, wet-dog shakes and scratching behavior produced by the cited agents are potently antagonized by several serotonin 5-$HT_{2A/C}$ receptor antagonists such as ketanserin, mianserin or ritanserin. Moreover, the selective 5-$HT_{2A/C}$ receptor agonist, 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI), can produce wet-dog shakes in rats (Schreiber et al., 1995; Darmani and Ahmad, 1999), and both head-twitch and scratching behaviors in mice (Darmani et al., 1990a,b) which are potently blocked by such 5-$HT_{2A/C}$ receptor antagonists.

In our preliminary studies intraperitoneal administration of a 2.5 mg/kg dose of SR 141716A in naive mice produced robust head-twitch and scratching behaviors. The initial purpose of the present study was to determine if the frequencies of the induced behaviors are dose related. Our second goal was to investigate whether SR 141716A-induced behaviors can be

blocked by: 1) the selective 5-$HT_{2A/C}$ receptor antagonist, trans-4-[(3Z)3-(2-dimethylaminoethyl)oxyimino-3-(2-fluorophenyl)propen-1-yl (SR 46349B) (Rinaldi-Carmona et al., 1992); 2) the cannabinoid agonist Δ^9-THC (Compton et al., 1992); 3) the AMPA/kianate receptor antagonist, 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) (Long et al., 1990); 4) the NMDA receptor antagonist, (±)-2-amino-3-phosphonopropionic acid (AP-3) (Collingridge and Lester, 1989); and 5) the tachykinin NK_1 receptor antagonist, (±)-(2S, 3S)-3-(2-methoxybenzylamino)-2-phenylpiperidine (CP 99, 944) (McLean et al., 1993).

Materials and methods

Animals and drugs

Male albino ICR mice (18–24 g) were used throughout the study. Animals were housed in groups of five on a 12L/12D cycle at a room temperature of 22 ± 1°C with ad lib supply of food and water. The following drugs were purchased from Research Biochemicals Inc. (Natick, MA): Δ^9-tetrahydrocannabinol (Δ^9-THC); CNQX (6-cyano-7-nitroquinoxaline-2,3-dione disodium); AP-3((±)-2–amino-3-phosphonopropionic acid). SR 141716A was a gift from Prof. B. R. Martin. SR 46349B was donated by Sanofi Recherche (Montpellier, France). CP 99, 944 was obtained from Pfizer Inc. (Groton, CT). Δ^9-THC and SR 141716A were dissolved in a 1 : 1 : 18 solution of ethanol, emulphor and 0.9% saline. Emulphor (EL-620, a polyoxyethylated vegetable oil, GAF Corporation, Linden, NJ) is currently available as ALKmulphor. Other drugs were dissolved in distilled water. All drugs were administered intraperitoneally (i.p.) at a volume of 0.1 ml/10 g of body weight. All animals received care according to the "Guide for The Care and Use of Laboratory Animals", DHSS Publication, revised, 1985. The facilities are certified by the American Association of Accreditation of Laboratory Care. The studies were approved by the Institutional Animal Care and Use Committee of KCOM.

Measurement of scratching and head-twitch behaviors

The scratch behavior is a rapid scratching movement of the head, neck or lateral area by either hind limb. The frequency of scratching episodes were scored by a multiple tally counter by a trained observer. A scratching episode produced by a particular hind limb consisted of 1 or more repetitive scratches with less than a 2-second in between. If the interval between consecutive scratches by a particular hind limb was greater than 2 seconds, the scratches were considered as separate episodes. If the scratches were produced by alternative hind legs, then each scratch was considered as a separate episode. The head-twitch response (HTR) in mice is analogous to wet-dog shakes in rats. It is a distinctive behavior and usually cannot be mistaken for other head movements, such as lateral head-shakes (lateral movement of head from side to side) or head-jerks (up and down jerking). The head-twitch frequency was also scored by a tally counter. Both the scratching and the head-twitch frequencies were recorded for 20 minutes immediately following administration of SR 141716A. The total mean score (±S.E.M.) for each behavior was then computed. In initial studies, the intensity of grooming behavior was also scored at 5-min intervals in the following intensity scale: absent (score 0), periodic (score 1), semicontinuous (score 2) or continuous (score 3). The grooming scores were collected over the 20-min observation period and the total cumulative mean score (±S.E.M.) was subsequently calculated.

Experimental protocols

To determine whether SR 141716A produces scratching, head-twitch and grooming behaviors in naive animals, mice were allowed to habituate to the test environment in plastic

cages (40 × 25 × 26 cm) lined with wood chippings for 20 min prior to treatment. Then, different groups of mice were injected intraperitoneally (i.p.) with varying doses of SR 141716A (0, 2.5, 5, 10 and 20 mg/kg, n = 6–7 per group). The frequency or intensity of the cited behaviors (mean ± SEM) for each mouse was individually scored for 20 min immediately following SR 141716A injection as was described earlier. For drug interaction studies, a 10 mg/kg dose of SR 141716A was used to induce scratching and head-twitch behaviors. Thus, at 0 min either corresponding vehicle or varying doses of the following drugs were injected intraperitoneally into different groups of mice: 1) the 5-$HT_{2A/C}$ receptor antagonist SR 46349B (0.1, 0.25, 1 and 3 mg/kg, n = 5–6 per group); 2) the cannabinoid agonist Δ^9-THC (10 and 20 mg/kg, n = 6 per group); 3) the AMPA/kianate receptor antagonist CNQX (5, 10 and 20 mg/kg, n = 6–7 per group); 4) the NMDA receptor antagonist AP-3 (4 and 10 mg/kg, n = 6–7 per group), and 5) the tachykinin NK_1 receptor antagonist CP 99, 994 (10 and 20 mg/kg, n = 6–8 per group). Twenty minutes later, each mouse was injected with a 10 mg/kg dose of SR 141716A and the frequencies of scratching and head-twitch behaviors (mean ± SEM) were recorded for the next 20 minutes as described above.

Statistical analysis

The scratching and head-twitch data were analyzed by a one-way analysis (ANOVA) of variance followed by Dunnett's t-test as posthoc analysis. The grooming data were analyzed by the Kruskal-Wallis nonparametric one-way analysis of variance and posthoc analysis by Dunn's multiple comparisons test. A p-value of <0.05 was necessary to achieve statistical significance. The ED_{50} (the effective agonist dose that produced 50% maximal response) and ID_{50} (the effective antagonist dose that attenuated response by 50%) were calculated by the use of a computerized program (Graph Pad InPlot, San Diego, CA).

Results

Intraperitoneal administration of SR 141716A produced both scratching (ED_{50} = 3.9 ± 1.4 mg/kg) and head-twitching (4.6 ± 1.2 mg/kg) behaviors in naive mice in a dose-dependent manner in the 20 min observation period following its injection (Fig. 1). One-way analysis of variance followed by Dunnett's t-test showed that relative to vehicle-treated control group, significant enhancements in scratching episodes (519, 554, 701 and 1194% increase over control respectively) occurred at 2.5, 5, 10 and 20 mg/kg doses of SR 141716A ($F(4,27) = 35.3$, $P < 0.0001$) (Fig. 1, top panel). There were also highly significant potentiations in HTR frequency at the cited doses of SR 141716A (335, 390 and 915% increase over control group respectively) ($F(4,27) = 15.8$, $P < 0.0001$) (Fig. 1, middle panel). However, relative to control group, SR 141716A failed to significantly alter the grooming behavior at any of the tested doses (Fig. 1, bottom panel).

In drug interaction studies, the 5-$HT_{2A/C}$ receptor antagonist, SR 46349B, dose-dependently attenuated both the SR 141716A-induced scratching (ID_{50} = 0.62 ± 1.95 mg/kg) and head-twitches (ID_{50} = 0.08 ± 1.3 mg/kg) (Fig. 2). Indeed, relative to the control group, significant reductions (56, 55 and 68%) in scratching frequency were observed at 0.25, 1 and 3 mg/kg doses of SR 46349B respectively ($F(4,22) = 6.98$, $P < 0.0009$) (Fig. 2, top panel). Thus, complete inhibition of scratching behavior was not achieved up to the highest tested dose of SR 46349B. On the other hand, the 5-$HT_{2A/C}$ receptor antagonist nearly completely blocked the ability of SR 141716A to produce

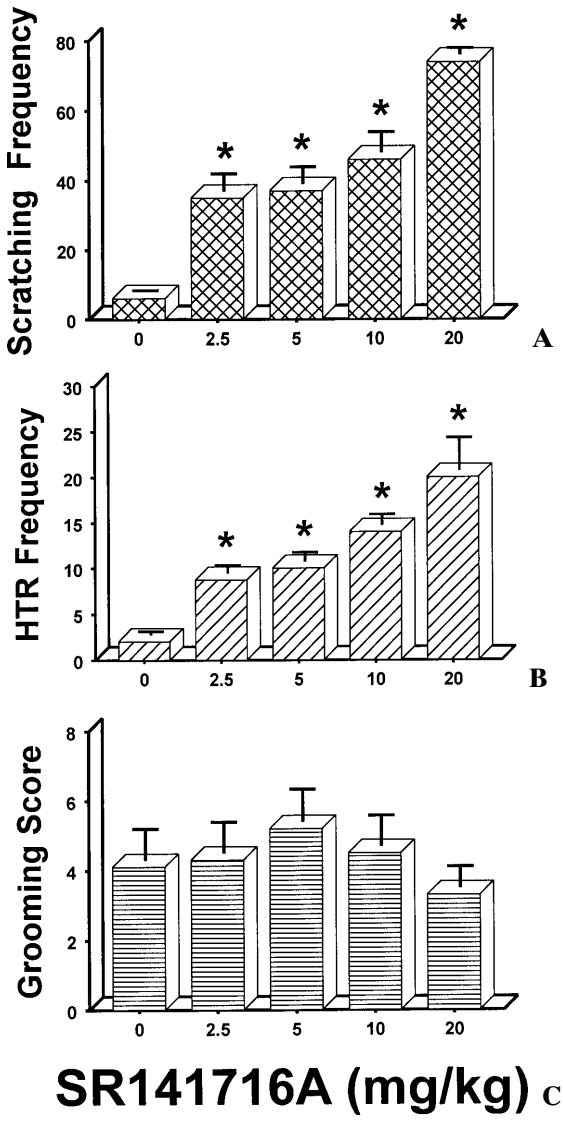

Fig. 1. The dose-response effects (mean ± SEM, n = 6–7) of the selective cannabinoid CB_1 receptor antagonist SR 141716A on the production of: scratchings (**A**), head-twitch response (HTR) (**B**) and grooming (**C**) in naive mice. The behaviors were observed simultaneously for 20 min immediately following injection of the cited doses of SR 141716A. *Significantly different from vehicle-treated control group by one-way analysis of variance followed by Dunnett's t-test at $p < 0.05$

head-twitches and significant reductions (51, 86, 84, 96%, respectively) were observed from the lowest tested dose (i.e. 0.1, 0.25, 1 and 3 mg/kg, respectively) of SR 46349B ($F(4,22) = 55.62$, $P < 0.0001$) (Fig. 2, lower panel).

The cannabinoid agonist Δ^9-THC at 10 and 20 mg/kg doses failed to significantly alter the SR 141716A-induced scratching behavior (Fig. 3, top panel). However, Δ^9-THC did attenuate the induced head-twitches, and a significant reduction (69%) relative to control group was observed at its

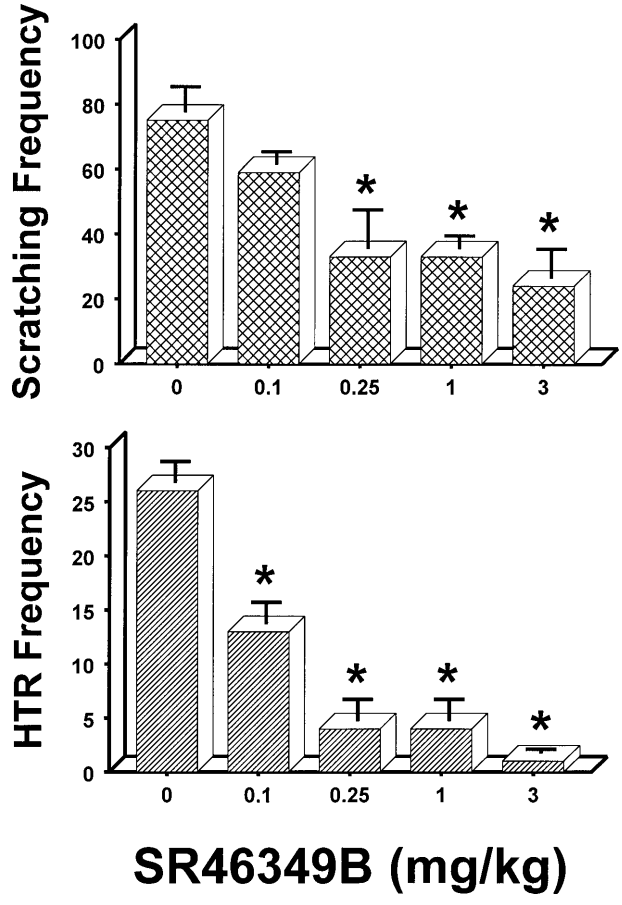

Fig. 2. Dose-dependent inhibitory effects of the selective 5-$HT_{2A/C}$ receptor antagonist, SR 46349B, on the frequencies of scratching and head-twitch response (HTR) behaviors induced by the selective cannabinoid CB_1 antagonist SR 141716A. The cited doses of SR 46349B (n = 5–6 per group) were injected 20 min prior to SR 141716A administration (10 mg/kg, i.p.). Data are presented as mean ± SEM (n = 5–6 per group) for the 20 min observation period following SR 141716A injection. *Significantly different from control at $p < 0.05$

20 mg/kg dose ($F(2,15) = 4.9$, $P < 0.02$) (Fig. 3, lower panel). The AMPA/kianate receptor antagonist CNQX attenuated both scratching and HTR behaviors produced by SR 141716A, however, neither behaviors were completely blocked (Fig. 4). Indeed, significant reductions (50 and 68%) in scratching episodes occurred at 10 and 20 mg/kg doses of CNQX ($F(3,22) = 13$, $P < 0.0001$) (Fig. 4, top panel). The frequency of SR 141716A-induced HTR was more sensitive to the inhibitory action of CNQX as it significantly reduced (60, 73 and 78%) the induced behavior at 5, 10 and 20 mg/kg doses ($F(2,22) = 28.4$, $P < 0.0001$) (Fig. 4, bottom panel). Furthermore, the ID_{50} dose of CNQX was significantly lower in inhibiting the frequency of HTR (3.7 ± 1.2) than the scratching behavior (10.9 ± 1.2 mg/kg). The NMDA receptor antagonist AP-3 failed to significantly alter the SR 141716A-induced

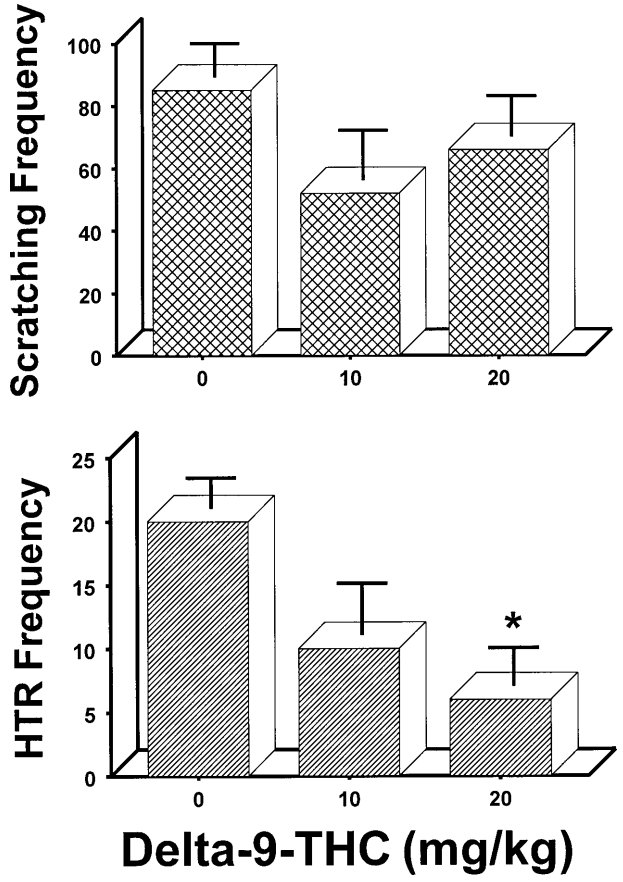

Fig. 3. The inhibitory action of delta-9-THC (Δ^9-THC) on the ability of selective cannabinoid CB_1 receptor antagonist SR 141716A to produce scratching and head-twitch response (HTR). The cited doses of Δ^9-THC were administered 20 min prior to injection of SR 141716A (10 mg/kg, i.p.). The frequencies of scratching and HTR behaviors (mean ± SEM, n = 6 per group) were recorded for 20 min following SR 141716A injection. *Significantly different from vehicle control at $p < 0.05$

head-twitch and scratching behaviors in mice (Fig. 5). The tachykinin NK_1 receptor antagonist, CP 99, 994, at 5 and 10 mg/kg doses significantly attenuated the frequencies of both scratching (45 and 64% reduction relative to control, respectively) and head-twitch (47 and 76% reduction relative to vehicle control, respectively) behaviors produced by SR 141716A (Fig. 6) [($F(2,17) = 10.4$, $P < 0.01$) and ($F(2,17) = 22.1$, $P < 0.01$) respectively].

Discussion

Several recent studies have shown that an acute injection of the cannabinoid CB_1 antagonist SR 141716A can induce scratching and head-twitches (or its behavioral homologue "the wet-dog shakes in rats) in both naive mice and chronically vehicle-exposed rodents (Cook et al., 1998; Aceto et al., 1998; Rubino et al., 1998). A significant enhancement in the head-twitch frequency

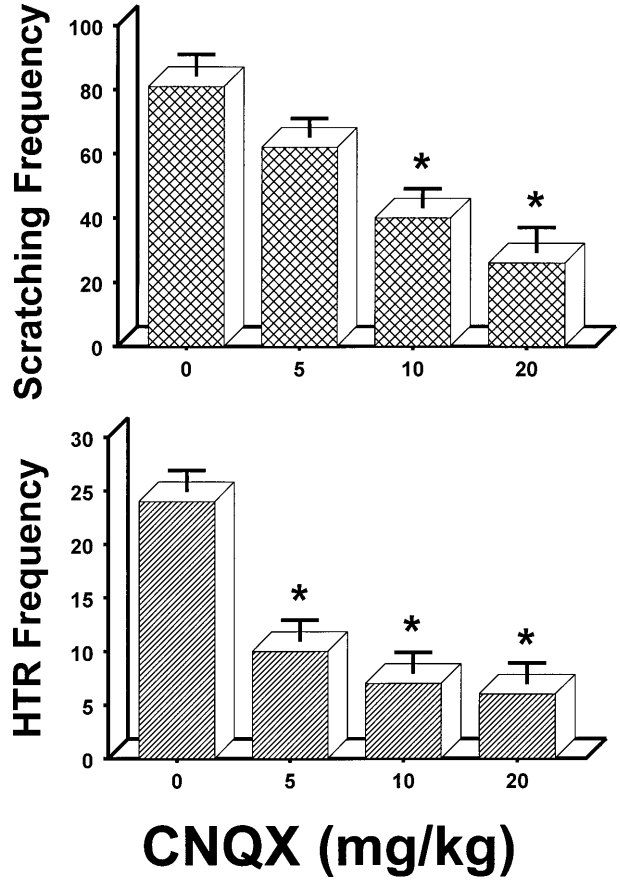

Fig. 4. Inhibition of SR 141716A-induced scratching and head-twitch response (HTR) behaviors by the AMPA/kainate receptor antagonist CNQX. The cited doses of CNQX (n = 6–7 per group) were administered 20 min prior to SR 141716A (10 mg/kg, i.p.) injection. The behaviors (mean ± SEM) were recorded for 20 min following SR 141716A injection. *Significantly different from vehicle control at $p < 0.05$

in naive mice has been reported to only occur after an intraperitoneal administration of 30 mg/kg dose of SR 141716A, whereas significant scratching episodes were apparent at its 10 mg/kg or greater doses (Cook et al., 1998). In the present study, the lowest tested dose of SR 141716A (2.5 mg/kg, i.p.) produced robust frequencies of both head-twitch and scratching behaviors in naive mice. The prime reason for the observed potency difference in these studies appears to be the relative young age of mice used in the present investigation. Indeed, in older animals lower frequencies of these behaviors are observed in response to administration of tachykinin-, glutamatergic-, or serotonergic-receptor agonists that induce such effects (Darmani et al., 1996; Darmani and Ahmad, 1999; Eble and Goodrich, 1987; Stoessl et al., 1993; Velisek et al., 1994). Thus, unlike the initial reports (Aceto et al., 1995; Tsou et al., 1995), there is substantial recent evidence that SR 141716A by itself can produce head-twitch and scratching behaviors in rodents.

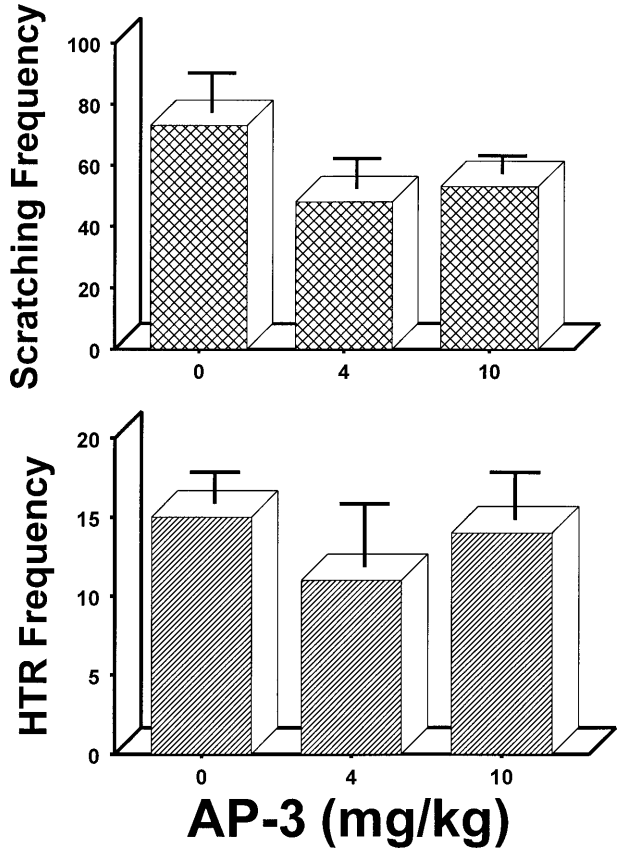

Fig. 5. The NMDA antagonist AP-3 had no significant effect on the ability of SR 141716A (10 mg/kg, i.p.) to produce scratching and head-twitch response (HTR) behaviors. The cited doses of AP-3 (n = 6–7 per group) were administered 20 min prior to SR 141716A administration

As yet no study has examined the receptor mechanism(s) by which SR 141716A-induced behaviors are elicited. As discussed in the introduction section, stimulation of several different neurotransmitter receptor systems (serotonin, tachykinin and excitatory amino acid) can precipitate these behaviors in rodents. Direct activation of these receptor systems by SR 141716A in producing scratching and head-twitch behaviors appear to be highly unlikely since it lacks affinity for the cited neurotransmitter receptors (Rinaldi-Carmona et al., 1994). Thus, indirect activation of one or more of the discussed neurotransmitter receptors is probably responsible for the behavioral activity of SR 141716A. Production of scratching and head-twitching by serotonergic-, glutamatergic- and tachykinin-receptor agonists ultimately involve direct or indirect activation of serotonergic 5-$HT_{2A/C}$ sites (Darmani et al., 1990a,b; Mjellem et al., 1993; Stoessl et al., 1987; Schreiber et al., 1995; Worms et al., 1981). Thus, in the present study, the ability of the selective 5-$HT_{2A/C}$ receptor antagonist SR 46349B to prevent SR 141716A-induced behaviors was first investigated. Pretreatment with SR 46349B potently reduced

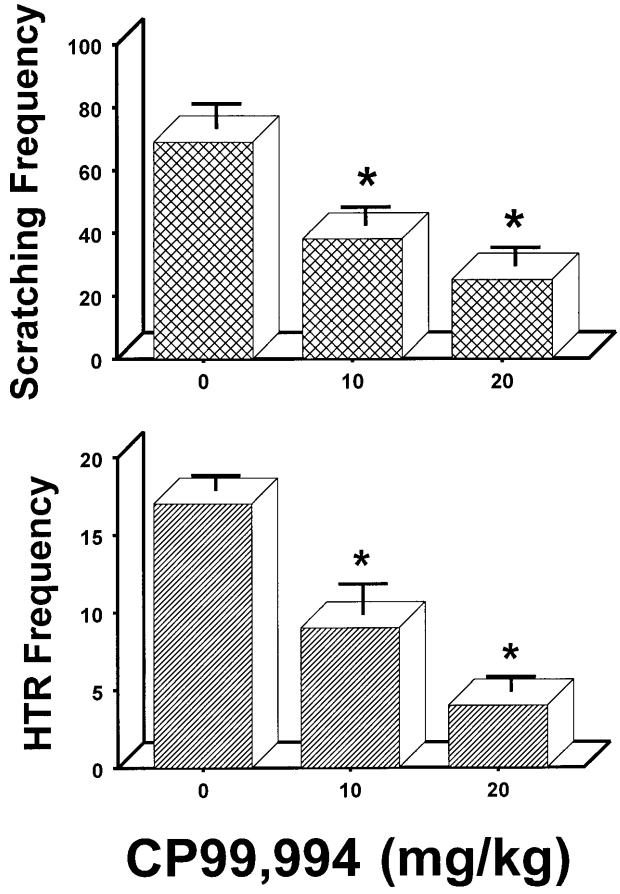

Fig. 6. The inhibitory actions of the tachykinin NK_1 antagonist CP 99, 994 on the ability of the selective cannabinoid CB_1 antagonist to induce scratchings and head-twitch responses (HTR). The cited doses of CP 99, 994 were administered 20 min prior SR 141716A (10 mg/kg, i.p.) injection. The frequencies of scratching and HTR behaviors (mean ± SEM, n = 6–8) were recorded for 20 min following SR 141716A injection. *Significantly different from vehicle control at $p < 0.05$

both the head-twitch (ID_{50} = 0.08 mg/kg) and scratching (0.6 mg/kg) behaviors induced by SR 141716A. However, although a near complete blockade of head-twitching was apparent at its 3 mg/kg dose, this relatively high dose of SR 46349B only partially (68%) prevented the ability of SR 141716A to induce the scratching behavior. Since SR 46349B has a greater affinity for the 5-HT_{2A} versus the 5-HT_{2C} sites (Rinaldi-Carmona et al., 1992), it is possible that the SR 141716A-induced head-twitch reflects indirect activation of 5-HT_{2A} sites, whereas the induced scratchings occur via indirect activation of 5-HT_{2C} sites. Overall, these results suggest that SR 141716A may indirectly potentiate serotonergic function by increasing its turnover in serotonergic terminal fields. In line with this notion, previous studies have shown that the cannabinoid agonist Δ^9-THC can reduce both whole brain 5-HT turnover (Sofia et al., 1971; Poddar et al., 1976), and 5-hydroxyindole acetic acid/serotonin (5-HIAA/5-HT) ratios in several but not all brain regions (Molina-

Holgado et al., 1993). Furthermore, in the present investigation, Δ^9-THC significantly but not completely blocked the ability of SR 141716A to induce head-twitches, however it failed to affect the scratching capacity of SR 141716A to a significant degree. It is possible that a larger dose of Δ^9-THC is required to fully block the induced head-twitches or scratchings since this cannabinoid acts as a partial agonist in several behavioral and biochemical assays (Burkey et al., 1997; Compton et al., 1992). Moreover, it should be noted that unlike SR 141716A, indirect (5-HT releasers and precursors) and nonselective direct 5-HT agonists produce head-twitches (or wet-dog shakes) and serotonin syndrome but not the scratching behavior following their peripheral administration (Review: Heal et al., 1992). On the other hand, selective 5-$HT_{2A/C}$ receptor agonists such as DOI induce both the head-twitch and scratching behaviors in mice (Darmani et al., 1990a,b), but produce wet-dog shakes and back muscle contractions (and not scratchings) in rats (Pranzatelli, 1990; Darmani and Ahmad, 1999).

There are two main glutamate receptor divisions comprising the ionotropic and metabotropic receptor families (Dingledine et al., 1999). The ionotropic glutamate receptors consist of NMDA, AMPA and kainate receptor subtypes. Systemic injection of kainate receptor selective agonists such as kainic acid and domoic acid result in production of both head-twitching (or wet-dog shakes) and scratching behaviors in rodents (Tasker et al., 1991; Velisek et al., 1994; Worms et al., 1981). In the present study, the AMPA/kainate receptor antagonist CNQX partially blocked (68–78%) both scratching and head-twitching behaviors produced by SR 141716A. This finding suggest a possible indirect role for glutamate in the behavioral actions of SR 141716A. Indeed, it is already known that cannabinoid agonists inhibit glutamate release in rat hippocampal cultures (Shen et al., 1996). Furthermore, SR 141716A-induced hyperalgesia can be attenuated by NMDA receptor antagonists (Richardson et al., 1998). The latter study proposes that inhibition of endogenous cannabinoid activity by SR 141716A results in glutamate release which subsequently activates NMDA receptors to produce analgesia. Thus, according to this notion, SR 141716A may produce its behavioral effects indirectly via potentiation of release of endogenous glutamate which subsequently would activate AMPA/kainate receptors to produce head-twitching and scratching behaviors. On the contrary, both Δ^9-THC and CNQX attenuate glutamatergic synaptic transmission (Shen and Thayer, 1999) and subsequently should block SR 141716A-induced behaviors. The glutamate NMDA receptor appears not be involved in the mediation of SR 141716A-induced HTR and scratching since its selective antagonist, AP-3, failed to affect the intensity of these behaviors. However, definite conclusions regarding the exact role of different glutamate receptors on SR 141716A-induced behaviors can only be made when the effects of several selective antagonists for each subtype of glutamate receptors are investigated.

The tachykinins constitute a family of neuropeptides comprising substance P (SP), neurokinin A (NKA) and neurokinin B (NKB) (Betancur et al., 1997). Tachykinins interact with at least three receptor subtypes termed NK_1, NK_2 and NK_3. Substance P binds preferentially to NK_1 receptor, whereas

neurokinin A (NKA) and neurokinin B (NKB) are preferred endogenous ligands for NK_2 and NK_3 receptors respectively. Substance P and 5-HT coexist in central neurons (Chan-Palay et al., 1978) and these agents increase each others turnover and release (Forchetti et al., 1982; Iverfeldt et al., 1986). Thus, it is not surprising that central administration of tachykinin agonists can induce a number of behaviors including scratching, head-twitching and serotonin syndrome (Eide, 1992; Ravard et al., 1994; Stoessl et al., 1987). In the present study, the NK_1 antagonist CP 94, 994 partially attenuated both the scratchings (64% reduction) and the head-twitches (76% reduction) produced by SR 141716A. These results suggest that the cannabinoid CB_1 receptor may functionally interact with the tachykinin neuronal system. Indeed, it is already known that chronic administration of Δ^9-THC increases mRNA levels of substance P (Mailleux et al., 1994).

It is possible that at the doses used the discussed agents may non-specifically reduce the induced HTR and ESR behaviours by general suppression of motor activity. However, several lines of evidence argue against this notion. Firstly, in general, drugs which attenuate locomotor activity and alter the discussed behaviors, usually affect the ESR at lower doses. In the present study, CNQX is more potent against the HTR instead of the ESR. Secondly, central administration of CNQX does not inhibit but promotes locomotor activity (Svensson et al., 1995; Burns et al., 1994), whereas systemic administration of high doses of this agent does not affect the amphetamine-induced locomotion (Mead and Stephens, 1999). Furthermore, CP 99, 994 only manages to block dopamine D_2 agonist-induced locomotion at 20 mg/kg or higher doses but can more potently prevent substance P-induced scratchings and wetdog shakes at lower doses (Rupniak and Jackson, 1994). In the present study the lowest tested dose of CP 99, 994 (10 mg/kg) significantly attenuated both the HTR and ESR equally. Although Δ^9-THC decreases spontaneous locomotion, this effect can be blocked by the cannabinoid CB_1 antagonist SR 141716A at relatively low doses (0.1–1 mg/kg) (Compton et al., 1996). Presently, only the 20 mg/kg dose of Δ^9-THC managed to significantly prevent the HTR but failed to affect the ESR produced by the 10 mg/kg dose of SR 141716A. Finally, although the NMDA antagonist, AP-3, had no effect on the induced HTR and ESR in the present study, it has been reported to suppress locomotor activity (Maginn et al., 1995). From the above discussion, it seems reasonable to suggest the cited drugs probably attenuate the HTR and ESR behaviors specifically via receptor interactions rather than general suppression of motor action.

In summary, systemic administration of SR 141716A produces both scratching and head-twitch behaviors in dose-dependent manner in naive mice. SR 141716A-induced behaviors seem to involve indirect potentiation of serotonergic, glutamatergic and tachykinin neurotransmitter systems.

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