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Behaviorally active doses of the CB_1 receptor antagonist SR 141716A increase brain serotonin and dopamine levels and turnover

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Abstract

Large doses (10–40 mg/kg) of the selective cannabinoid CB_1 receptor antagonist, SR 141716A, produce the head-twitch response (HTR) and scratching in rodents and vomiting in the least shrew (*Cryptotis parva*). Agents that increase brain serotonin (5-HT) levels induce the HTR in rodents, whereas enhancements in either brain 5-HT or dopamine concentrations can lead to production of emesis in vomiting species. The present study was undertaken to demonstrate whether large doses of SR 141716A can (1) induce the HTR and scratching in the least shrew and (2) cause concurrent biochemical changes in brain 5-HT and dopamine concentrations. SR 141716A (0, 1, 5, 10, 20 and 40 mg/kg ip) administration induced the HTR, scratching and vomiting. The HTR effect was bell shaped with a maximum frequency occurring at the 20 mg/kg SR 141716A dose, whereas the scratching and vomiting behaviors displayed dose-dependent effects. The selective 5-$HT_{2A/C}$ receptor antagonist, SR 46349B (0, 0.1, 0.25, 1, 3 and 6 mg/kg ip), differentially attenuated all SR 141716A (20 mg/kg)-induced behaviors because the HTR was relatively more potently and completely blocked. In the shrew forebrain, SR 141716A (20 and 40 mg/kg ip) caused dose- and time-dependent increases in the levels of 5-HT and dopamine and the concentrations of their major metabolites [5-hydroxyindole acetic acid (5-HIAA), 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanilic acid (HVA)] and the turnover of both monoamines. Although the effects of SR 141716A on brainstem concentrations of both monoamines and their metabolites were not always consistent, the CB_1 antagonist did increase the turnover of both 5-HT and dopamine. The present findings suggest that the mechanism and the neurochemical substrate for SR 141716A-induced HTR and scratching behaviors is enhancement of 5-HT release, whereas increased release of 5-HT and dopamine probably contributes to the production of emesis.

Keywords: SR 141716A; SR 46349B; Vomiting; Head-twitch response; Scratching; Vomit; Cannabinoid CB_1 receptor; Serotonin; 5-HIAA; Dopamine; DOPAC; HVA; 5-HT_{2A} receptor; Release

1. Introduction

Exogenous cannabinoids (plant derived and synthetic) and endocannabinoids mainly produce their effects via cannabinoid CB_1 and CB_2 receptors (Howlett et al., 2002; Pertwee, 1999). The CB_1 receptor is preferentially located in the brain, spinal cord and peripheral neurons, whereas the CB_2 receptor is mainly found on peripheral nonneuronal tissues. One important function of CB_1 receptors seems to be the modulation of release of several neurotransmitters at specific central and peripheral sites. Indeed, structurally diverse cannabinoid receptor agonists [such as Δ^9-tetrahydrocannabinol (Δ^9-THC), CP 55,940, WIN 55,212-2 or anandamide] have been shown to inhibit neurotransmitter release [acetylcholine, dopamine, norepinephrine, serotonin (5-HT), etc.] (Howlett et al., 2002; Schlicker and Kathman, 2001). The presynaptic CB_1 receptor seems to modulate the release of the cited neurotransmitters from diverse neuronal systems because the selective cannabinoid CB_1 receptor antagonist, SR 141716A, counters the inhibitory effect of cannabinoid agonists.

In addition to preventing the effects of cannabinoid agonists (Pertwee, 1997, 1999), SR 141716A produces a number of biochemical and behavioral effects when administered alone. For example, at normal pharmacological doses (1–5 mg/kg), SR 141716A increases GI motility and intestinal transit (Calignano et al., 1997; Colombo et al., 1998; Izzo et al., 1999), reduces food intake and enhances memory as well as produces conditioned place preference (reviewed in Chaperon and Thiebot, 1999). At moderate to large doses (5–40 mg/kg), it produces head twitching and scratching in

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rodents (Aceto et al., 1995; Cook et al., 1998; Darmani and Pandya, 2000; Rubino et al., 1998) and vomiting in the least shrew (*Cryptotis parva*) (Darmani, 2001a). The downstream neurotransmitters by which SR 141716A produces these behaviors are not yet fully elucidated. However, it is known that at low to moderate doses (0.3–5 mg/kg) SR 141716A increases (1) the release of acetylcholine in hippocampal microdialysates (Gessa et al., 1998) as well as potentiating electrically induced release of [^{14}C] acetylcholine in hippocampal slices (Gifford et al., 1997), (2) the extracellular dialysate concentrations of norepinephrine in rat hypothalamus and hippocampus (Tzavara et al., 2001) as well as potentiating the glutamate-mediated release of norepinephrine from hippocampal slices (Kathmann et al., 1999) and (3) the hypothalamic microdialysate concentrations of the dopamine metabolites 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanilic acid (HVA) (Tzavara et al., 2001). However, at such normal pharmacological doses, SR 141716A does not appear to alter either the release or the CNS concentration of 5-HT (5-hydroxytryptamine) or its major metabolite 5-hydroxyindole acetic acid (5-HIAA) (Arévalo et al., 2001; Nakazi et al., 2000; Tzavara et al., 2001). The latter biochemical findings are not consistent with the discussed behavioral studies in which moderate to large doses of SR 141716A produce serotonergically mediated behaviors in mice and rats and vomiting in the least shrew, which could be due to activation of release of 5-HT or dopamine. The prefrontal cortex (Willins and Meltzer, 1997) and brainstem (Heal et al., 1992; Veyrat-Follet et al., 1997) are among the important loci in the brain that produce the latter behaviors. The aim of the present study was (1) to demonstrate whether large doses of SR 141716A can produce the serotonergically mediated head-twitch response (HTR) and scratching in the least shrew and (2) to identify the possible neurochemical substrates responsible for the production of these behaviors. Thus, the exposure time effects of large doses of SR 141716A (20 and 40 mg/kg) were investigated on the turnover and concentrations of 5-HT, dopamine and their respective major metabolites (5-HIAA, DOPAC and HVA) in the least shrew forebrain and brainstem by means of high-performance liquid chromatography with electrochemical detection (HPLC-ECD).

2. Materials and methods

2.1. Animals

Male and female shrews (*C. parva*) were bred and housed in the animal facilities of the Kirksville College of Osteopathic Medicine. Shrews weighing 4–6 g (45–70 days old) were used throughout the study. The animals were kept on a 14:10-h light/dark cycle at a humidity-controlled environment in a room temperature of 22 ± 1 °C with ad libitum supply of food and water. All animals received care according to the Guide for the Care and Use of Laboratory Animals (Department of Health and Human Services Publication, revised 1985). All of the procedures used in this study were approved by the Institutional Animal Care and Use Committee of the college.

2.2. Behavioral studies

Previously, we have shown that large doses of SR 141716A produce emesis in shrews (Darmani, 2001a) and the HTR and scratching in mice (Janoyan et al., 2002). In the current study, we investigated whether SR 141716A can also produce the HTR and scratching behaviors in the least shrew. The HTR and scratching in the least shrew are similar behaviors to those observed in mice. The HTR is a distinctive head-twitching behavior that cannot be mistaken for other head movements such as lateral headshakes (lateral movement of the head from side to side) or head jerks (up and down jerking of the head). The scratching behavior is a rapid scratching of the head, neck or loin area by either hind limb. A scratching episode produced by a particular hind limb consisted of one or more repetitive scratches with less than a 2-s interval between scratches. When the interval between scratches was greater than 2 s, the scratches were considered separate episodes. In addition, when alternative hind legs produced scratches, they were considered separate episodes. The HTR and scratch frequencies (means ± S.E.M.) were recorded using a multiple tally counter by a trained observer.

To determine the dose–response effects of SR 141716A on the production of HTR, scratching and vomiting behaviors in the least shrew, animals were transferred to the experimental room and were allowed to acclimate for at least 1 h prior to experimentation. To habituate the shrews to the test environment, each animal was randomly selected and transferred to a 20 × 16 × 21 cm clean, clear plastic cage and offered four mealworms (*Tenebrio* sp.) 30 min prior to drug administration. Different groups of shrews were injected intraperitoneally (ip) with varying doses of SR 141716A (0, 1, 5, 10, 20 and 40 mg/kg, $n = 8–10$ per group), and the frequency of induced behaviors was recorded for 30 min following injection. A dose of 20 mg/kg SR 141716A was shown to produce robust frequencies of HTR and scratching behaviors. To investigate whether the induced behaviors have a serotonergic origin, the ability of the selective 5-$HT_{2A/C}$ receptor antagonist SR 46349B to prevent SR 141716A (20 mg/kg)-induced HTR and scratching was investigated. Because the 20 mg/kg dose of SR 141716A also produces robust episodes of vomiting (Darmani, 2001a), the possible antiemetic effect of SR 46349B was also concurrently investigated. Thus, at time zero, different groups of shrews received either vehicle ($n = 13$) or varying doses of SR 46349B (0.1, 0.25, 1, 3 and 6 mg/kg ip, $n = 7–11$ per group). Thirty minutes later, each shrew was injected with a 20 mg/kg dose of SR 141716A and the frequencies (mean ± S.E.M.) of HTR, scratching and vomiting were recorded for the next 30 min.

2.3. Neurotransmitter studies

Prior to experimentation, shrews were habituated to the laboratory environment as described for the behavioral studies. A large number of shrews were then injected intraperitoneally with either vehicle ($n=34$) or SR 141716A (20 and 40 mg/kg, $n=31-32$ per group). Then, subgroups ($n=7-10$) of each treatment were sacrificed by rapid decapitation at 15, 30, 60 and 120 min after injection. Brains were rapidly removed and dissected on an ice-cold Petri dish, and the forebrain and brainstem were immediately frozen on dry ice and stored at -80 °C until analysis. Each forebrain and brainstem sample was respectively homogenized (tissue tearer, setting 5 for 15 s) in 1 or 2 ml ice-cold 0.2 M perchloric acid containing 0.3 M EDTA. A 0.1 ml portion of each homogenate was removed for protein analysis. The protein concentration was determined by the use of BCA protein assay kit (Pierce, Rockford, IL). Then, homogenates were centrifuged at $40,000 \times g$ for 30 min at 4 °C to precipitate protein. The supernatant was filtered through a 0.2 μm nylon filter in a microcentrifuge tube at $1500 \times g$ for 10 min at 4 °C. A 10 μl aliquot of each filtered supernatant was assayed for the presence of monoamines and their metabolites.

Monoamines and their metabolites were analyzed by HPLC-ECD based on a modification of the method of Rutter et al. (1995). Separation of 5-HT, dopamine and their corresponding metabolites (5-HIAA, DOPAC and HVA) from other electroactive compounds was achieved on a 10 cm × 3.2 mm RP-C18 column (ODS 3 μm packing; BAS, West Lafayette, IN) and a mobile phase containing 0.15 M chloroacetic acid, 0.12 M sodium hydroxide, 0.18 mM EDTA, 1.1 mM sodium octane sulfonic acid and 5% acetonitrile. The monoamines and their metabolites were separated and measured through the cited column via a Shumadzu L-ECD-6A detector connected to a Shimadzu LC-10 AD pump and a C-R7Ae chromatopac integrator (Kyoto, Japan). The glassy carbon working electrode was set at a potential of 650 mV relative to an Ag/AgCl reference electrode. The detection limits for dopamine and 5-HT were 2 and 5 pg, respectively, based on a signal-to-noise ratio of 3:1. Peak heights of unknowns were compared with the mean peak heights of a 200 pg standard solution (containing 5-HT, dopamine, 5-HIAA, HVA and DOPAC), which were run daily after every fifth tissue supernatant sample.

2.4. Statistical analysis

The HTR and scratching behaviors were analyzed by one-way analysis of variance (ANOVA) followed by Fisher's Post Hoc Test. The frequency of emesis data was analyzed by the Kruskal–Wallis (KW) nonparametric one-way ANOVA and post hoc analysis by Dunn's Multiple Comparisons Test. The incidence of emesis (number of shrews vomiting) was analyzed by the Fisher's Exact Test to determine whether there were differences between groups. When appropriate, pairwise comparisons were also made by this method. Neurochemical data were initially analyzed by two-way ANOVA with drug dose and exposure time as factors. Fisher's LSD Test was used as post hoc test. When dose and exposure factors significantly interacted but a significant dose or time effect was not observed by the two-way ANOVA for either of these factors, then appropriately a one-way ANOVA with Fisher's test was performed for that factor. For each statistical test, a P value of $<.05$ was necessary to achieve statistical significance.

2.5. Drugs

Both SR 141716A and SR 46349B were generously donated by Sanofi-Synthelabs Recherche (Montpellier, France). SR 141716A was initially dissolved in a 1:1:18 solution of ethanol/emulphor/0.9% saline to twice the stated concentrations. These drug concentrations were further diluted by the addition of an equal volume of saline. SR 46349B was dissolved in distilled water. All drugs were administered intraperitoneally at a volume of 0.1 ml/10 g body weight.

3. Results

3.1. Behavioral studies

Intraperitoneal administration of SR 141716A in the least shrew produced two serotonergically mediated motor behaviors, the HTR and scratching [$F(5,47)=6.08$, $P<.0002$ and $F(5,47)=6.66$, $P<.0001$, respectively] (Fig. 1). The HTR frequency was bell shaped, and the Fisher's Post Hoc Test showed a significant increase only at the 20 mg/kg dose (Fig. 1A). The scratching behavior did not show a bell-shaped dose–response effect, and significant increases in the frequency were apparent from the 5–40 mg/kg doses of SR 141716A (Fig. 1B). In order to determine whether these induced behaviors are 5-HT_{2A} receptor mediated, the inhibitory effects of varying doses (0, 0.1, 0.25, 1, 3 and 6 mg/kg) of the selective 5-$HT_{2A/C}$ receptor antagonist, SR 46349B, were investigated on the SR 141716A-induced behaviors in shrews. A relatively high dose of 20 mg/kg SR 141716A was chosen to produce robust frequencies of HTR and scratching. One-way ANOVA indicated that SR 46349B reduced both induced behaviors [$F(5,46)=4.4$, $P<.002$ and $F(5,46)=5.4$, $P<.0005$, respectively] (Fig. 2). The cited doses of SR 46349B attenuated the HTR frequency by 42%, 79%, 93%, 84% and 96%, respectively (Fig. 2A). However, significance was obtained from the 0.25 mg/kg dose. The scratching behavior was relatively more resistant to inhibition, and only the highest tested dose of SR 46349B (6 mg/kg) significantly reduced the behavior by 78% (Fig. 2B). We

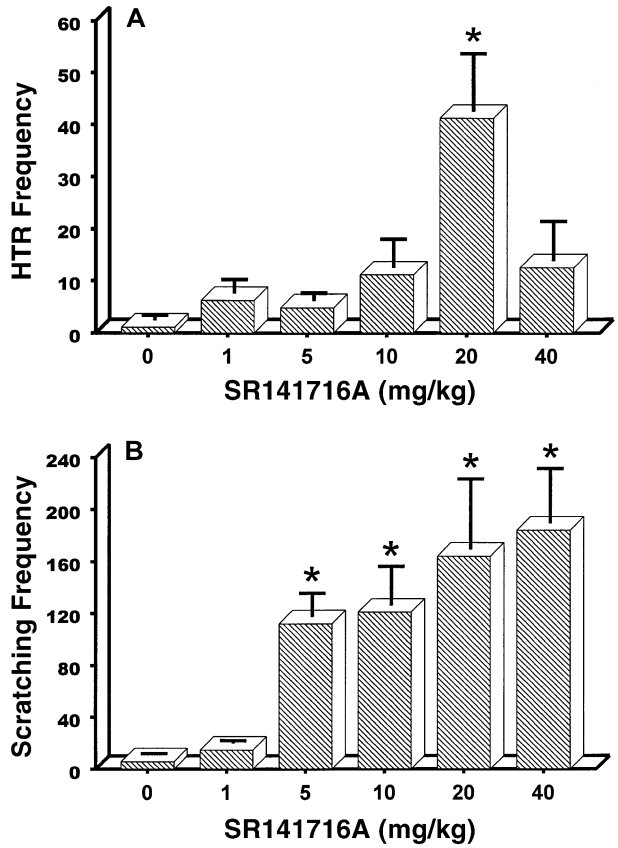

Fig. 1. The dose–response effects (means ± S.E.M.) of the selective cannabinoid CB_1 receptor antagonist on the production of HTR (A) and scratching behavior (B) in shrews. These behaviors were observed simultaneously for 30 min immediately following injection of the cited doses of SR 141716A. Data are presented as means ± S.E.M. * Significantly different from vehicle-treated control group by one-way ANOVA followed by Fisher's test.

have previously shown that large doses of SR 141716A (10–40 mg/kg) can induce dose-dependent increases in the percentage of shrews vomiting and the vomiting frequency (Darmani, 2001a). In the present study, the cited doses of SR 141716A also caused vomiting (data not shown). Pretreatment with SR 46349B also significantly reduced both the number of shrews vomiting and the frequency of vomits produced by a 20 mg/kg dose of SR 141716A [$\chi^2(5,49) = 18.95$, $P < .002$ and $KW(5,49) = 17.49$, $P < .004$, respectively) (Fig. 3). Dunn's Multiple Comparisons Test showed that the highest tested dose of SR 46349B (6 mg/kg) significantly reduced the vomit frequency by 93% (Fig. 3A). Likewise, Fisher's Exact Test showed that SR 46349B significantly reduced the percentage of shrews vomiting by 79% at this dose (Fig. 3B).

3.2. Neurotransmitter studies

Two-way ANOVA of shrew forebrain 5-HT concentration for SR 141716A dose and exposure time resulted in highly significant differences between doses [$F(2,84) = 15.06$, $P < .000003$] and among exposure periods [$F(3,84) = 5.27$, $P < .002$] (Fig. 4A). There was also a significant interaction between treatments and exposure periods [$F(6,84) = 2.5$, $P < .03$]. Fisher's LSD Multiple Comparisons Post Hoc Test showed that SR 141716A caused significant dose- and time-dependent increases in forebrain 5-HT concentrations, i.e., 52% and 149% increase relative to corresponding vehicle controls at 30 min for the 20 and 40 mg/kg treatment groups, respectively, and a 54% increase at 60 min for the 40 mg/kg dose (Fig. 4A). In addition, the 5-HT level at the 30-min exposure period for the 40 mg/kg SR 141716A dose was also significantly greater than its corresponding 5-HT concentration at the 15-min exposure period. Two-way ANOVA also revealed similar enhancements in forebrain 5-HIAA concentrations for SR 141716A doses [$F(2,84) = 22.7$, $P < .000001$] and exposure periods [$F(3,84) = 6.13$, $P < .008$] (Fig. 4B). A significant interaction between doses and exposure periods was also observed [$F(6,84) = 5.73$, $P < .00005$]. Fisher's test indicated a significant increase (180% relative to corresponding vehicle control) in 5-HIAA concentration at the 30-min exposure time for the 20 mg/kg dose of SR 141716A.

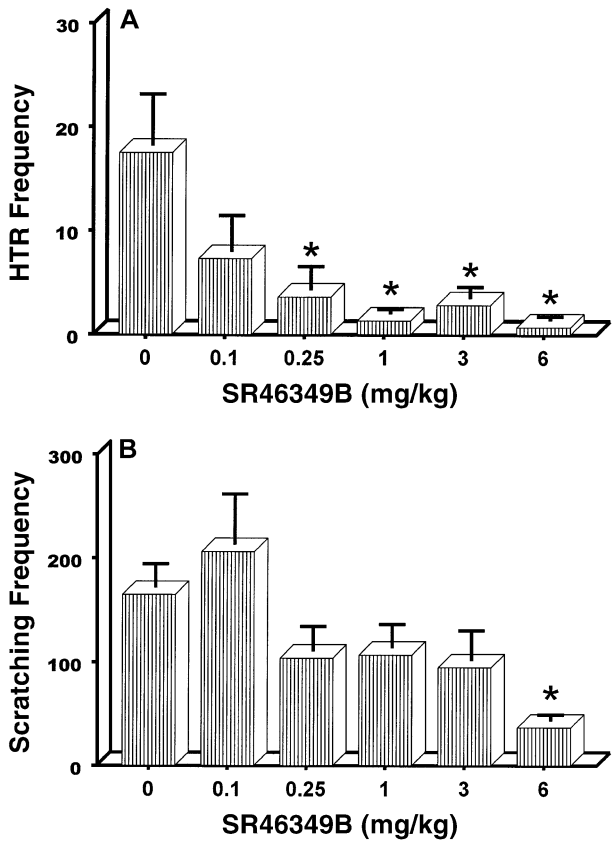

Fig. 2. Inhibition of SR 141716A-induced HTR and scratching by the cited doses of the selective 5-$HT_{2A/C}$ antagonist SR 46349B. The different doses of SR 46349B were administered 30 min prior to the administration of a 20 mg/kg dose of SR 141716A, and the cited behaviors were recorded for the next 30 min. Data are presented as means ± S.E.M. * Significantly different from corresponding control group by one-way ANOVA followed by Fisher's test.

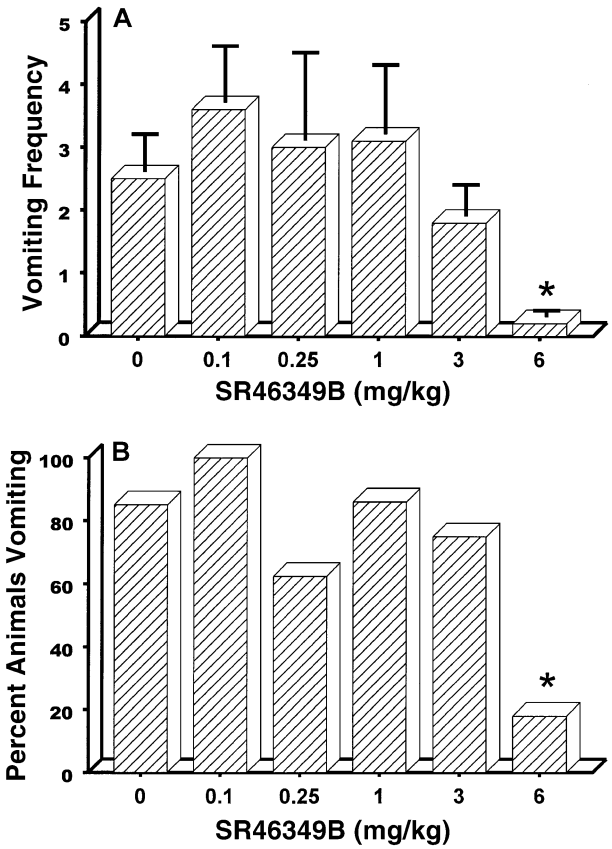

Fig. 3. The ability of the selective 5-$HT_{2A/C}$ receptor antagonist SR 46349B in attenuating the vomiting frequency (A) and the percentage of shrews vomiting (B) in response to a 20 mg/kg dose of the CB_1 antagonist SR 141716A. * Significantly different from corresponding vehicle either by the KW one-way ANOVA test followed by Dunn's Multiple Comparisons Test or Fisher's Exact Test.

The 40 mg/kg dose caused significant increases (356% and 94% relative to their corresponding vehicle controls) at 30- and 60-min exposure periods. Furthermore, 5-HIAA concentrations for the 20 mg/kg dose at the 30- and 120-min exposure periods and for 40 mg/kg dose at 30- and 60-min exposure times were greater than their corresponding concentrations at the 15-min period. The ratio of monoamine metabolite concentration to that of the parent neurotransmitter has already been used as an index of monoamine turnover in studies involving administration of cannabinoid agonists and antagonists (Arévalo et al., 2001). In the present study, the concentration ratios of 5-HIAA/5-HT, DOPAC/dopamine and HVA/dopamine were used as respective indices of 5-HT and dopamine turnover. The forebrain 5-HT turnover [as expressed by 5-HIAA concentration ÷ 5-HT concentration] exhibited dose-dependent [$F(2,84)=4.18$, $P<.02$] but not time-dependent effects ($P>.05$) (Fig. 4C). There was also a significant interaction between doses and exposure periods [$F(6,84)=4.43$, $P<.0006$]. Fisher's test revealed that SR 141716A caused significant ($P<.05$) and dose-dependent increases in 5-HT turnover (74.4% at 20 mg/kg and 96% at 40 mg/kg) at the 30-min exposure time and a 33% ($P<.05$) increase during the 60-min exposure for the 40 mg/kg dose. In order to show that the discussed interaction between doses and exposure periods does not mask any possible significant exposure-dependent effect of SR 141716A on 5-HT turnover, one-way ANOVAs were performed separately for SR 141716A doses across the 15-, 30-, 60- and 120-min exposure times. These analyses also failed to show significance.

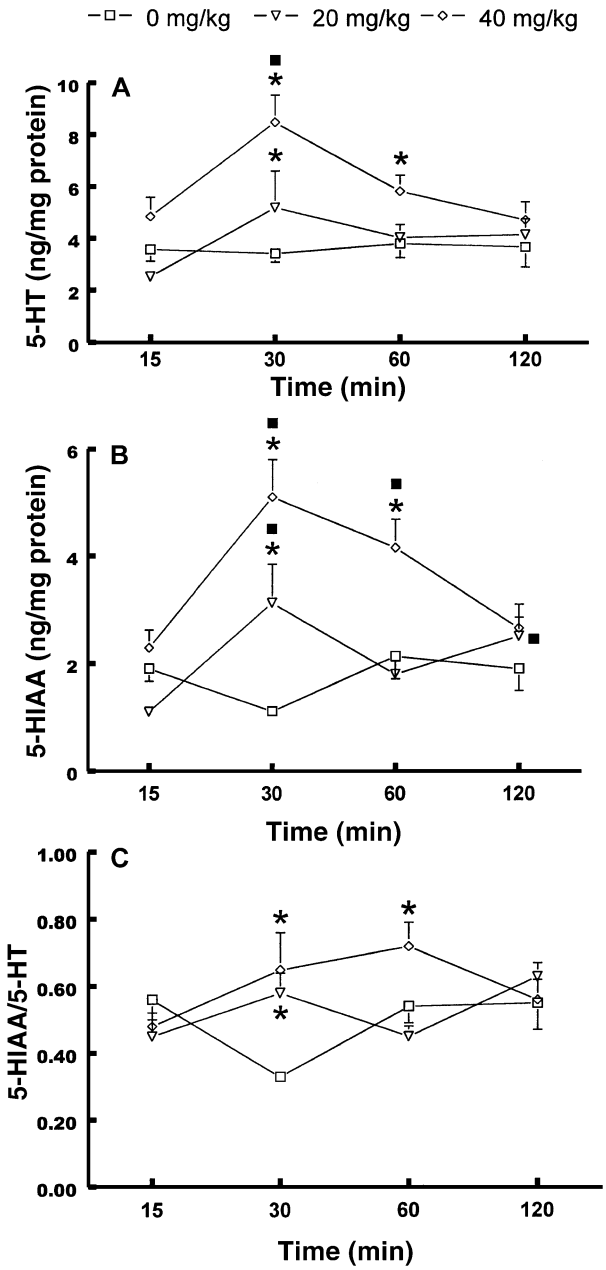

Fig. 4. Time–response effects of different doses of SR 141716A (0, 20 and 40 mg/kg) on the concentrations of 5-HT (A) and its major metabolite 5-HIAA (B) and 5-HT turnover (C) in the least shrew forebrain. * Significantly different at corresponding times from vehicle control at $P<.05$. ■Significantly different from corresponding 15-min values at $P<.05$.

The brainstem 5-HT function was less responsive to the effects of SR 141716A (Fig. 5). Indeed, two-way ANOVA failed to show either dose- or exposure-dependent effect on the 5-HT level, but there was a strong interaction between these factors [$F(6,84)=3.62$, $P<.003$] (Fig. 5A). Subsequent one-way ANOVAs followed by Fisher's test on different doses of SR 141716A revealed that the 40 mg/kg dose caused a 48% decrease [$F(2,21)=11.4$, $P<.0005$] in hindbrain 5-HT concentration at the 30-min exposure period, whereas the 20 mg/kg dose significantly increased the 5-HT level (80%) at the 120-min period [$F(2,19)=5.1$, $P<.02$] (Fig. 5A). Likewise, two-way ANOVA for brainstem 5-HIAA levels showed a significant interaction between SR 141716A doses and exposure periods [$F(6,84)=3.7$, $P<.003$] but no significant dose or exposure effects (Fig. 5B). However, subsequent one-way ANOVAs followed by Fisher's test showed that the 40 mg/kg SR 141716A dose did cause a 33% decrease in the 5-HIAA level at the 30-min exposure period. As for the case of both 5-HT and 5-HIAA concentrations, two-way ANOVA also failed to show significant dose or exposure effects for 5-HT turnover but again exhibited a significant interaction between SR 141716A doses and exposure times [$F(6,84)=3.52$, $P<.004$] (Fig. 5C). However, follow-up one-way ANOVAs showed significant increases (48% and 27%) in 5-HT turnover at the 30-min exposure period by the 20 and 40 mg/kg doses of SR 141716A, respectively.

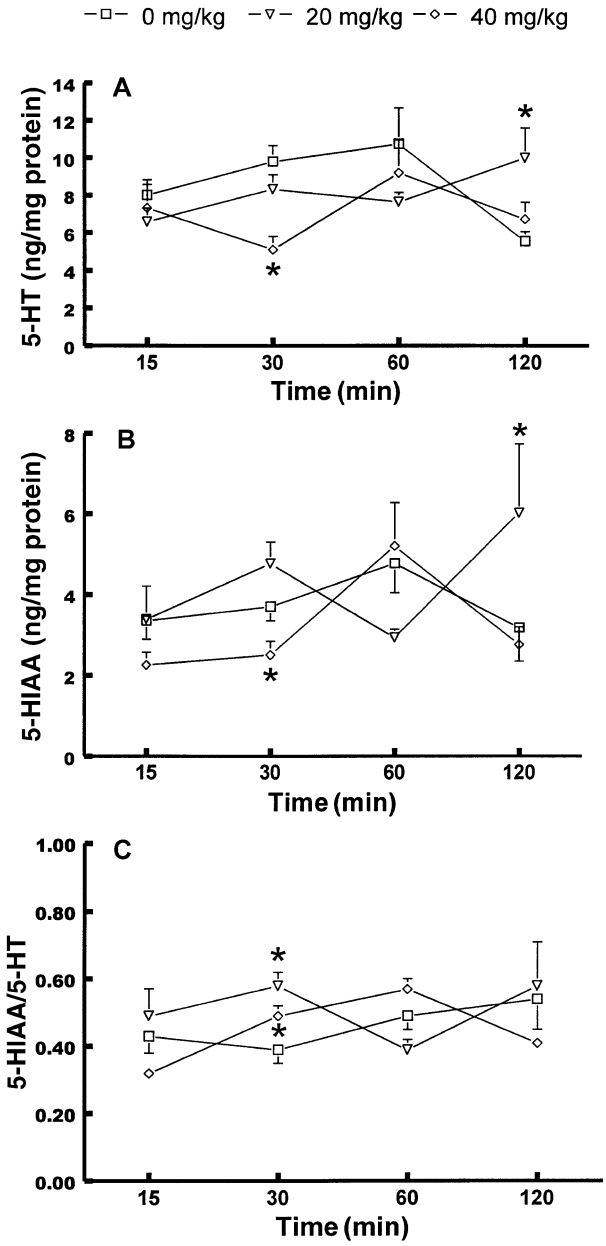

Fig. 5. Time–response effects of varying doses of SR 141716A (0, 20 and 40 mg/kg) on the concentrations of 5-HT (A) and its major metabolite 5-HIAA (B) and 5-HT turnover (C) in the least shrew brainstem. * Significantly different at corresponding times from vehicle control at $P<.05$.

Regarding forebrain dopamine levels, two-way ANOVA showed that SR 141716A caused dose-dependent [$F(2,84)=6.23$, $P<.003$] but not time-dependent effects (Fig. 6A). Furthermore, no significant interaction was observed among these factors. Fisher's test indicated that significant and dose-dependent increases [60% (20 mg/kg) and 101% (40 mg/kg) relative to corresponding vehicle-treated controls] occurred in dopamine levels at the 60-min exposure period only. SR 141716A administration also caused dose- and time-dependent increases in DOPAC levels in the shrew forebrains [$F(2,84)=5.72$, $P<.005$ and $F(3,84)=6.3$, $P<.0007$, respectively] (Fig. 6B). There was also a significant interaction between these factors ($P<.000003$). Fisher's test showed that, relative to their corresponding vehicle-treated controls, significant increases (346% and 69%) in DOPAC levels, respectively, occurred at the 30- and 60-min exposure periods for the 40 mg/kg SR 141716A treatment dose. The effect of the 20 mg/kg dose of SR 141716A was more complex as it caused a 48% ($P<.05$) decrease in DOPAC concentration at the 15-min period and a 203% ($P<.05$) increase following the 30-min exposure period. The discussed increases in DOPAC levels were also significantly greater than their corresponding levels at the 15-min period. Although two-way ANOVA showed that SR 141716A produced time-dependent increases in dopamine turnover (as expressed by DOPAC concentration ÷ dop-dopamine concentration [$F(3,84)=10.16$, $P<.000009$], the dosage effect failed to attain significance ($P=.08$) probably because of the significant interaction observed among these factors [$F(3,84)=10.24$, $P<.000001$] (Fig. 6C). Thus, one-way ANOVAs with Fisher's test were performed, which revealed that at the 30-min exposure period, SR 141716A dose-dependently and significantly increased [111% (20 mg/kg) and 219% (40 mg/kg) forebrain dopamine turnover [$F(2,22)=15.17$, $P<.001$]. Two-way ANOVA showed that SR 141716A administration caused both dose- and time-dependent increases in shrew forebrain levels of the other major dopamine metabolite HVA [$F(2,84)=10.24$, $P<.0001$ and $F(3,84)=4.19$, $P<.008$, respectively] (Fig. 7A). There

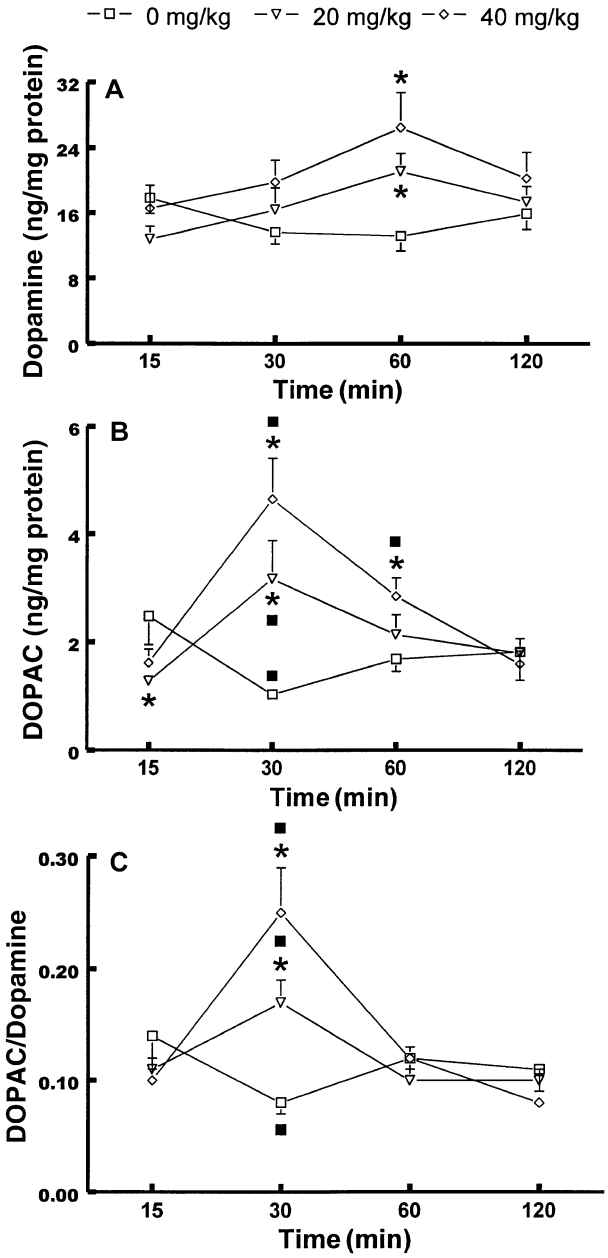

Fig. 6. Time–response effects of different doses of SR 141716A (0, 20 and 40 mg/kg) on the concentrations of dopamine (A) and its metabolite DOPAC (B) and dopamine turnover (C) in the least shrew forebrain. * Significantly different at corresponding times from vehicle control at $P<.05$. ■Significantly different from corresponding 15-min values at $P<.05$.

were no significant interaction between these factors ($P=.09$). Fisher's test showed that the 20 mg/kg dose of SR 141716A significantly increased (73% relative to vehicle control) HVA level at the 30-min exposure period, whereas the 40 mg/kg dose caused significant potentiations (180% and 73% relative to corresponding vehicle-treated controls) at the 30- and 60-min exposure periods. In addition, these effects were significantly greater than their corresponding effects at the 15-min exposure time. SR 141716A also caused dose- and time-dependent increases in dopamine forebrain turnover (as expressed by HVA concentration ÷ dopamine concentration) [$F(2,84)=3.23$, $P<.05$; $F(3,84)=5.03$, $P<.003$] (Fig. 7B). There was no significant interaction among doses and exposure periods ($P=.08$). Fisher's test indicated that the 40 mg/kg dose of SR 141716A caused a significant increase in dopamine turnover (45% relative to vehicle control) at the 30-min exposure time, which is also significantly different from its corresponding 15-min exposure period.

Unlike in the shrew forebrain, two-way ANOVA failed to show that SR 141716A significantly affected brainstem dopamine concentration probably because of a significant interaction between doses and exposure periods [$F(6,84)=3.45$, $P<.004$] (Fig. 8A). However, follow-up one-way ANOVAs revealed that relative to the corresponding vehicle-treated group, the 40 mg/kg dose of SR 141716A significantly decreased dopamine level by 49% at the 30-min exposure time [$F(2,22)=12.7$, $P<.0002$] (Fig. 8A). Moreover, the 20 mg/kg dose at the 30-min exposure period significantly increased dopamine levels relative to its corresponding 15-min exposure time [$F(3,26)=6.72$, $P<.002$]. Likewise, two-way ANOVA did not show significant effects either on brainstem DOPAC levels (Fig. 8B) or

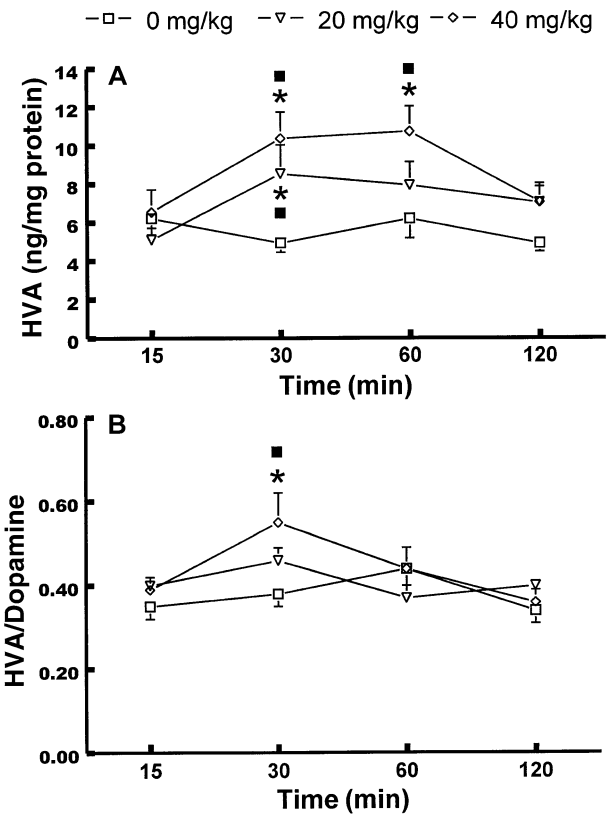

Fig. 7. Time–response effects of varying doses of SR 141716A (0, 20 and 40 mg/kg) on the concentration of dopamine metabolite HVA (A) and dopamine turnover (B) in the least shrew forebrain. * Significantly different at corresponding times from vehicle control at $P<.05$. ■Significantly different from corresponding 15-min values at $P<.05$.

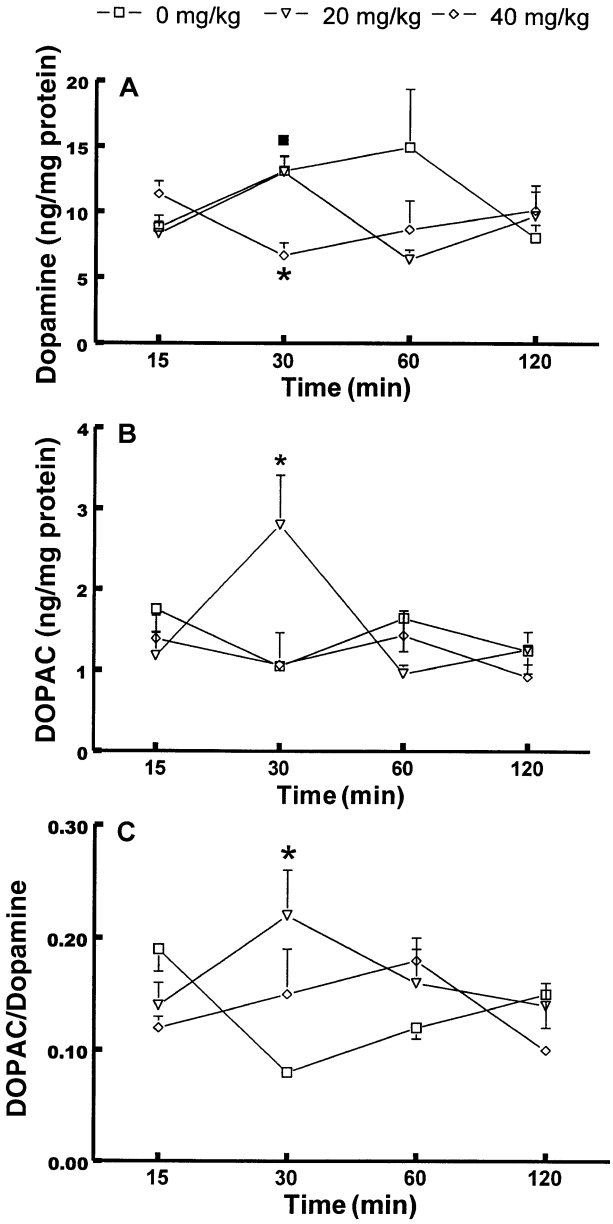

Fig. 8. Time–response effects of different doses of SR 141716A (0, 20 and 40 mg/kg) on the concentrations of dopamine (A) and its metabolite DOPAC (B) and dopamine turnover (C) in the least shrew brainstem. * Significantly different at corresponding times from vehicle control at $P<.05$. ■Significantly different from corresponding 15-min values at $P<.05$.

on dopamine turnover (as expressed as DOPAC concentration ÷ dopamine concentration) (Fig. 8C) but indicated strong interactions between respective SR 141716A doses and exposure periods [$F(6,84)=4.58$, $P<.0005$; $F(6,84)=4.93$, $P<.0003$]. However, follow-up one-way ANOVAs with Fisher's test indicated that the 20 mg/kg dose of SR 141716A significantly increased DOPAC level (167%) [$F(2,22)=7.02$, $P<.004$] (Fig. 8B) and dopamine turnover (142%) [$F(2,21)=4.41$, $P<.03$] (Fig. 8C) at the 30-min exposure period. Regarding the other metabolite of dopamine, two-way ANOVA indicated significant exposure effects for both HVA concentration [$F(3,84)=5.44$, $P<.002$] (Fig. 9A) and dopamine turnover (as expressed by HVA concentration ÷ dopamine concentration [$F(3,84)=19.15$, $P<.000001$] (Fig. 9B) but no significant effect among SR 141716A doses. There were also significant interactions [$F(6,84)=3.81$, $P<.002$; $F(6,84)=3.14$, $P<.008$] between doses and exposure periods for both HVA levels and dopamine turnover. Fisher's test following two-way ANOVA showed that the 20 mg/kg dose of SR 141716A significantly increased HVA brainstem levels at 30 min relative to its 15-min exposure period, and both 20 and 40 mg/kg doses of the antagonist significantly potentiated dopamine turnover at 60-min exposure period relative to their corresponding 15-min treatment periods. Follow-up one-way ANOVAs with Fisher's test indicated that (1) at 30-min exposure time, the 20 mg/kg SR 141716A dose significantly increased (30%), while the 40 mg/kg dose decreased (28%), brainstem HVA levels [$F(2,22)=9.17$, $P<.001$] (Fig. 9A) and (2) both doses of SR 141716A increased dopamine turnover (27.5% and 38%, respectively) at the 30-min exposure period [$F(2,22)=7.7$, $P<.003$], whereas the 40 mg/kg dose also enhanced dopa-

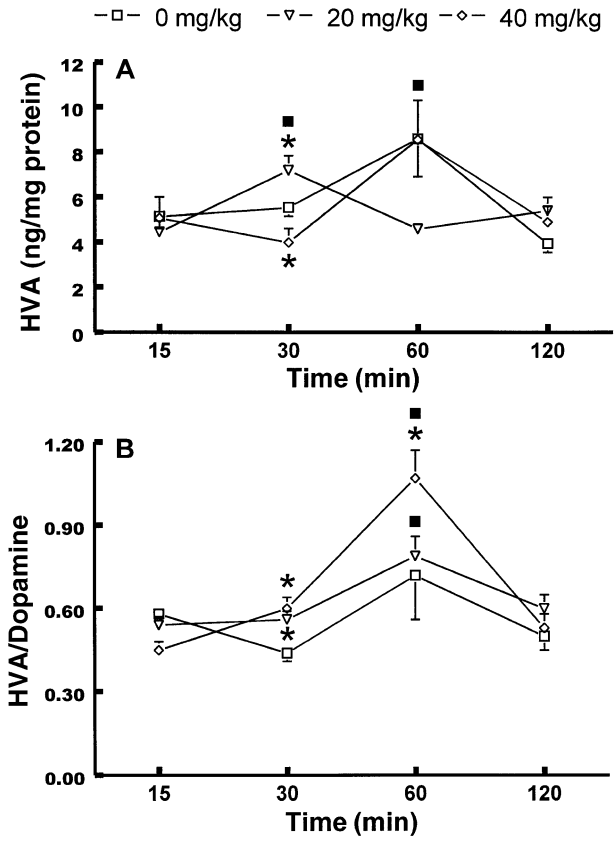

Fig. 9. Time–response effects of SR 141716A (0, 20 and 40 mg/kg) on the concentration of dopamine metabolite HVA (A) and dopamine turnover (B) in the least shrew brainstem. * Significantly different at corresponding times from vehicle control at $P<.05$. ■Significantly different from corresponding 15-min values at $P<.05$.

mine turnover at the 60-min exposure period [$F(2,21) = 3.6$, $P < .05$] (Fig. 9B).

4. Discussion

As discussed in Section 1, a number of studies have shown that peripheral administration of moderate to large doses of the CB_1 receptor antagonist SR 141716A produce the HTR and scratching behaviors in a dose-dependent fashion in drug-naive rodents. In addition, both the selective 5-HT_{2A} receptor antagonist SR 46349B and the structurally diverse cannabinoid agonists prevent these SR 141716A-induced behaviors in a dose-dependent fashion in mice (Darmani and Pandya, 2000). Likewise, in the present study, intraperitoneal injection of SR 141716A produced both the HTR and the scratching behaviors in the least shrew. However, the HTR effect was bell shaped, whereas the induced scratching behavior tended to display a dose-dependent pattern. As reported for mice (Darmani and Pandya, 2000), the tested doses of SR 141716A in the least shrew produced more robust frequencies of scratching than HTR. Moreover, relative to the SR 141716A-induced scratching, the induced HTR was more potently and completely blocked by the selective 5-$HT_{2A/C}$ antagonist SR 46349B in both species. These findings suggest that both the HTR and the scratching behaviors produced by SR 141716A in the least shrew are also 5-HT_{2A} receptor-mediated events. In addition to producing these serotonergic-mediated behaviors, large doses of SR 141716A can produce vomiting in the least shrew in a dose-dependent fashion, which can be potently blocked by structurally diverse cannabinoid agonists (Darmani, 2001a). In the present study, SR 141716A did produce significant emesis at doses greater than 10 mg/kg (data not shown). Furthermore, the largest tested dose of SR 46349B also significantly blocked the ability of SR 141716A to induce vomiting in the least shrew, which may imply a role for 5-HT_{2A} receptors in vomiting. Although it is generally accepted that among the 14 or more recognized subtypes of 5-HT receptors, serotonergic 5-HT_3 and 5-HT_4 receptors are involved in vomiting (Veyrat-Follet et al., 1997), more recent evidence suggest that DOI produces emesis in the pigeon via the activation of 5-HT_{2A} sites (Wolff and Leander, 2000), which seem to support the current findings. In addition, the chemotherapeutic agent cisplatin produces emesis via release of 5-HT (Veyrat-Follet et al., 1997), which both 5-HT_3 antagonists and structurally diverse cannabinoid agonists prevent in a dose-dependent manner in this and other vomiting species (Darmani, 2001b,c; Darmani, 1998; Veyrat-Follet et al., 1997).

Because SR 141716A lacks affinity for 5-HT receptors as well as other receptor systems involved in emesis and in the modulation of HTR and scratching (Rinaldi-Carmona et al., 1994), a direct action of the CB_1 antagonist on the production of these behaviors can be ruled out. To ascertain an indirect mode of action for SR 141716A, we investigated whether the CB_1 antagonist could increase the concentration or turnover of 5-HT and/or dopamine in the least shrew brain. Both monoamines can induce emesis via the brainstem (Veyrat-Follet et al., 1997), whereas 5-HT produces the HTR and scratching via serotonergic circuits involving the frontal cortex, brainstem and spinal cord (Heal et al., 1992; Willins and Meltzer, 1997). Thus, large groups of shrews were exposed to either vehicle or behaviorally effective doses of SR 141716A (20 and 40 mg/kg) and were subsequently sacrificed at increasing time intervals (i.e., 15, 30, 60 and 120 min postinjection). Relative to the brainstem, the shrew forebrain appeared to be a better substrate for the effects of SR 141716A on monoamine function. Indeed, SR 141716A dose- and time-dependently increased forebrain concentrations of 5-HT (52–192%) and dopamine (203–346%) and turnover of these monoamines. The maximal increase in 5-HT levels occurred at the 30-min exposure period, while dopamine levels were maximally affected at the 60-min postinjection period. The onset of effect of SR 141716A on forebrain levels of both monoamines required at least 30-min exposure and lasted for at least 1 h because a significant effect was not observed either at the 15- or 120-min exposure periods. The degree and time pattern of increase in forebrain concentration of the 5-HT metabolite 5-HIAA as well as the 5-HT turnover mirrored the changes observed in forebrain 5-HT levels. However, relative to the discussed changes in forebrain dopamine concentrations, significant increases in the levels of dopamine metabolites (DOPAC and HVA) and dopamine turnover (expressed as concentrations of either DOPAC ÷ dopamine or HVA ÷ dopamine) occurred earlier (at the 30-min exposure period). Moreover, the effect of 40 mg/kg dose of SR 141716A on the levels of dopamine metabolites persisted up to 60 min. Although this dose of SR 141716A significantly reduced both brainstem 5-HT and 5-HIAA concentrations, both doses of the CB_1 antagonist (20 and 40 mg/kg) increased brainstem 5-HT turnover at the 30-min exposure time. Likewise, while brainstem levels of dopamine and its metabolite HVA were attenuated by the 40 mg/kg dose at 60 min, the 20 mg/kg dose of SR 141716A increased the concentrations of both DOPAC and HVA at the 30-min exposure period. Moreover, brainstem dopamine turnover was increased by both doses of SR 141716A. The discussed decreases in brainstem concentrations of both monoamines are probably due to negative feedback pathways involving short or long loop projections arising from the observed overactivity of corresponding dopaminergic and serotonergic terminal fields (Koeltzow et al., 1998; Paris and Cunningham, 1994). Such regional variation in brain dopamine function in response to SR 141716A administration has already been noted. For example, electrophysiological studies have shown that SR 141716A can increase the firing rate of dopamine A9, but not A10, neurons (Gueudet et al., 1995). Moreover, neurotransmitter studies indicate that SR 141716A does not affect dopamine release from rat striatal slices (Cadogan et al., 1997) or the shell of the nucleus

accumbens (Alonso et al., 1999) but can increase the level of its metabolites in the anterior hypothalamus (Tzavara et al., 2001). The present neurochemical and behavioral findings support the notion that large doses of SR 141716A induce the HTR and scratching behaviors via enhancements in 5-HT release and turnover, while the induced vomiting may involve increases in the synaptic functional indices of both monoamines as evidenced by their increased levels and turnover. The proposed hypothesis is further strengthened by published observations that increased synaptic activity following 5-HT precursor (or releaser) loading can induce both HTR and vomiting (Heal et al., 1992; Ryan et al., 1991), whereas pretreatment with the dopamine precursor, L-DOPA, leads to vomiting only (Koller, 2000). Whether the monoamine release effects of high doses of SR 141716A in the present study is CB_1 receptor mediated is uncertain because such large doses of SR 141716A can also induce noncannabinoid effects (Sim-Selley et al., 2001).

In summary, the present data provide evidence that (1) intraperitoneal administration of large doses of the selective cannabinoid CB_1 receptor antagonist SR 141716A induces serotonergically mediated behaviors (the HTR and scratching) as well as vomiting in the least shrew, (2) high behaviorally active doses of SR 141716A (20–40 mg/kg) increase shrew forebrain levels of 5-HT and dopamine and the concentrations of their major metabolites (5-HIAA, DOPAC and HVA, respectively) as well as the turnover of both monoamines in a dose- and time-dependent manner. Although SR 141716A-induced changes in the levels of both monoamines and their metabolites in the shrew brainstem were not always consistent, the turnovers of both monoamines were dose- and time-dependently increased by the cited doses of SR 141716A. (3) It appears that the ability of SR 141716A to induce HTR and scratching is due to enhanced 5-HT release and turnover, whereas the induced vomiting may involve functional enhancement of both monoamines in their corresponding synapses.

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