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Central and peripheral mechanisms contribute to the antiemetic actions of delta-9-tetrahydrocannabinol against 5-hydroxytryptophan-induced emesis

Nissar A. Darmani*, Jane C. Johnson

Department of Pharmacology, Kirksville College of Osteopathic Medicine, A.T. Still University of Health Sciences, 800 West Jefferson Street, Kirksville, MO 63501, USA

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Abstract

Delta-9-tetrahydrocannabinol (delta-9-THC) prevents cisplatin-induced emesis via cannabinoid CB_1 receptors. Whether central and/or peripheral cannabinoid CB_1 receptors account for the antiemetic action(s) of delta-9-THC remains to be investigated. The 5-hydroxytryptamine (5-HT = serotonin) precursor, 5-hydroxytryptophan (5-HTP), is an indirect 5-HT agonist and simultaneously produces the head-twitch response (a centrally mediated serotonin $5\text{-}HT_{2A}$ receptor-induced behavior) and emesis (a serotonin $5\text{-}HT_3$ receptor-induced response, mediated by both peripheral and central mechanisms) in the least shrew (*Cryptotis parva*). The peripheral amino acid decarboxylase inhibitor, carbidopa, prevents the conversion of 5-HTP to 5-HT in the periphery and elevates 5-HTP levels in the central nervous system (CNS). When administered i.p. alone, a 50 mg/kg dose of 5-HTP failed to induce either behaviour while its 100 mg/kg dose produced robust frequencies of both head-twitch response and emesis. Pretreatment with carbidopa (0, 10, 20 and 40 mg/kg) potentiated the ability of both doses of 5-HTP to produce the head-twitch response in a dose-dependent but bell-shaped manner, with maximal potentiation occurring at 20 mg/kg carbidopa. Carbidopa dose-dependently reduced the frequency of 5-HTP (100 mg/kg)-induced emesis, whereas the 10 mg/kg dose potentiated, and the 20 and 40 mg/kg doses suppressed the frequency of vomits produced by the 50 mg/kg dose of 5-HTP. The peripheral and/or central antiemetic action(s) of delta-9-THC (0, 1, 2.5, 5, 10 and 20 mg/kg) against 5-HTP (100 mg/kg)-induced head-twitch response and emesis were investigated in different groups of carbidopa (0, 10 and 20 mg/kg) pretreated shrews. Irrespective of carbidopa treatment, delta-9-THC attenuated the frequency of 5-HTP-induced head-twitch response in a dose-dependent manner with similar ID_{50} values. Although delta-9-THC also reduced the frequency of 5-HTP-induced emesis with similar ID_{50s}, at the 5 mg/kg delta-9-THC dose however, 5-HTP induced significantly less vomits in the 10 and 20 mg/kg carbidopa-treated groups relative to its 0 mg/kg control group. Moreover, increasing doses of carbidopa significantly shifted the inhibitory dose–response effect of delta-9-THC in protecting shrews from 5-HTP-induced emesis to the left. Relatively, a large dose of delta-9-THC (20 mg/kg) was required to significantly reduce the number of vomits produced by direct acting serotonergic $5\text{-}HT_3$ receptor agonists, serotonin and 2-methylserotonin. Low doses of delta-9-THC (0.1–1 mg/kg) nearly completely prevented 2-methylserotonin-induced, centrally mediated, head-twitch and ear-scratch responses. The results indicate that delta-9-THC probably acts pre- and postsynaptically to attenuate emesis produced by indirect and direct acting $5\text{-}HT_3$ receptor agonists via both central and peripheral mechanisms. In addition, delta-9-THC prevents 5-HTP-induced head-twitch and emesis via cannabinoid CB_1 receptors since the CB_1 receptor antagonist, SR 141716A [*N*-piperidino-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methylpyrazole-3-carboxamide], countered the inhibitory actions of an effective dose of delta-9-THC against both behaviours.

Keywords: Delta-9-THC; 5-HTP; 5-HT; Carbidopa; SR 141716A; Head-twitch; Ear-scratch; Central; Peripheral; Cannabinoid CB_1 receptor

1. Introduction

To date two subtypes of cannabinoid receptors, CB_1 and CB_2, are cloned and their pharmacology is well investigated (Howlett et al., 2002; Pertwee, 1999). Cannabinoid CB_1 receptors are primarily found on central and peripheral neurons where one of their functions is to inhibit neurotransmitter release. Cannabinoid CB_2 receptors are mainly found in peripheral tissues such as immune cells. Both cannabinoid receptors are coupled through G proteins to several signal transduction mechanisms.

Recent studies from this laboratory have shown that well investigated representatives of structurally diverse cannabi-

* Corresponding author. Tel.: +1-660-626-2326; fax: +1-660-626-2728.
E-mail address: ndarmani@atsu.edu (N.A. Darmani).

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noids {[delta-9-tetrahydrocannabinol] (delta-9-THC); [1α,2β-(*R*)-5α]-(−)-5-(1,1-dimethyl)-2-[5-hydroxy-2-(3-hydroxypropyl) cyclohexylphenol] (CP 55,940) and [*R*(±)-[2,3-dihydro-5-methyl-3-[(morpholinyl] pyrolol [1,2,3) de]-1,4-benzoxazin-yl]-(1-naphthalenyl) methanone mesylate] (WIN 55,212-2)} prevent cisplatin-induced emesis via stimulation of the cannabinoid CB_1 receptor since their antiemetic activity was countered by low to intermediate doses of the selective cannabinoid CB_1 receptor antagonist SR 141716A [*N*-piperidino-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methylpyrazole-3-carboxamide] (Darmani, 2001b,c; Darmani et al., 2003a). At such doses, another cannabinoid CB_1 receptor antagonist, AM 251 [*N*-(piperidin-1-yl)-5-(4-iodophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide], was also shown to reverse the antiemetic activity of WIN 55,212-2 against emesis produced by morphine 6-glucuronide (Van Sickle et al., 2001). At large doses (>10 mg/kg), SR 141716A by itself produces emesis in a dose-dependent manner and the emetic effect was blocked by cannabinoid CB_1/CB_2 receptor agonists (Darmani, 2001a, 2002). The cannabinoid CB_1 receptor, its endogenous ligands (endocannabinoids) and the major enzyme responsible for the degradation of endocannabinoids, fatty acid amide hydrolase, are found in brainstem (Darmani et al., 2003a; Van Sickle et al., 2001; Herkenham et al., 1991; Bisogno et al., 1999; Sugiura et al., 2002; Izzo et al., 2001) as well as the gastrointestinal tract and its enteric nervous system (Pertwee, 2001; Pinto et al., 2002). These central and peripheral loci control both the initiation and coordination of gastrointestinal motility as well as emesis (Krowicki and Hornby, 1995; Veyrat-Follet et al., 1997). Recent reviews have concluded that the inhibitory effects of systemically administered cannabinoid agonists on gastric emptying and intestinal transit are largely mediated by peripheral cannabinoid CB_1 receptors on enteric nerves, whereas brainstem cannabinoid CB_1 receptors play a relatively lesser role (Pertwee, 2001; Pinto et al., 2002). Based on their findings that both the cannabinoid CB_1 receptor and fatty acid amide hydrolase are present in several emetic loci in the dorsal vagal complex of the ferret, Van Sickle et al. (2001) concluded that cannabinoids inhibit emesis via a central mechanism. On this basis, cannabinoids may also produce their antiemetic activity through peripheral mechanisms since the cannabinoid CB_1 receptor, endcannabinoids and fatty acid amide hydrolase are also found in the gastrointestinal tract.

In order to investigate the possible peripheral and central roles of the antiemetic action of delta-9-THC, we utilized the ability of the serotonin (5-hydroxytryptamine = 5-HT) precursor 5-hydroxytryptophan (5-HTP) to induce vomiting. Following systemic administration, 5-HTP (but not 5-HT) can enter the central nervous system (CNS) and undergo decarboxylation to form 5-HT both in the periphery and the CNS (Hartvig et al., 1993; Endo, 1985). The peripheral amino acid decarboxylase inhibitor carbidopa reduces serotonin levels in the periphery by preventing the conversion of 5-HTP to 5-HT and thus elevates the 5-HTP bioavailability to the CNS leading to increased synthesis and release of 5-HT in the brain (Endo, 1985; Gartside et al., 1992). Since 5-HT cannot pass the blood–brain barrier, the actions of systemically administered 5-HTP in the presence of a peripherally effective dose of carbidopa is considered an index of central effects of serotonin (Goodrich et al., 1989; Endo, 1985; Handley and Singh, 1986). Indeed, carbidopa potentiates the ability of 5-HTP to produce centrally mediated serotonergic 5-HT_{2A} receptor-induced head-twitch response in mice (Handley and Singh, 1986; Endo, 1985) while reducing the 5-HTP-induced hypoglycemia which is peripherally mediated (Endo, 1985). Current literature suggests that serotonin 5-HT_3 receptor antagonists prevent emesis via blockade of both peripheral (Gidda et al., 1995; Fukui et al., 1992) and central (Gidda et al., 1995; Yoshida et al., 1992) serotonin 5-HT_3 receptors. We have previously shown that direct-acting selective serotonin 5-HT_{2A} receptor agonists induce the head-twitch response (Darmani et al., 1994; Zhao, 1996), while selective serotonin 5-HT_3 receptor agonists produce emesis (Darmani, 1998) in shrews. The purpose of this study was to: (1) determine the optimum peripheral effective dose of carbidopa needed to potentiate centrally mediated 5-HTP-induced head-twitch response which would be then utilized for comparing the relative contribution of central and central + peripheral emetic components of 5-HTP; (2) ascertain the relative ability of delta-9-THC to prevent 5-HTP-induced head-twitch response in the absence and presence of the peripherally effective dose of carbidopa; (3) investigate the central versus central + peripheral components of antiemetic actions of delta-9-THC respectively in the absence and presence of the peripherally effective dose of carbidopa against 5-HTP-induced vomiting; (4) determine whether the inhibitory actions of delta-9-THC against 5-HTP-induced head-twitch response and vomiting are mediated via cannabinoid CB_1 receptors; and (5) investigate the relative ability of delta-9-THC to inhibit emesis produced by the systemic administration of direct-acting nonselective (serotonin) and selective (2-methylserotonin) serotonin 5-HT_3 receptor agonists which respectively act via peripheral and peripheral/central mechanisms to produce emesis since 2-methylserotonin, but not serotonin, can pass the blood–brain barrier (Glennon et al., 1992).

2. Materials and methods

2.1. Animals and drugs

Shrews (*Cryptotis parva*) were bred and maintained in the animal facilities of the Kirksville College of Osteopathic Medicine. Both male and female shrews (4–6 g, 35–60 days old) were used throughout the study. The feeding and maintenance of shrews are fully described elsewhere (Darmani, 1998, 2001b). Delta-9-tetrahydrocannabinol, 5-hydroxytryptophan, serotonin and 2-methylserotonin were

purchased from Sigma/RBI (St. Louis, MO). SR 141716A [*N*-piperidino-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methylpyrazole-3-carboxamide] was generously donated by Sanofi Recherche (Montpellier, France). Carbidopa was donated by Merck, Sharp and Dohme (Westpoint, PA). Delta-9-THC and SR 141716A were initially dissolved to twice the stated drug concentrations in a 1:1:18 solution of ethanol/emulphor/0.9% saline. The drug concentrations were then diluted by the addition of an equal volume of saline. This procedure was necessary because the 1:1:18 vehicle mixture can by itself cause emesis in up to 20% of the animals. The final vehicle mixture induced emesis in up to 10% of shrews. 5-Hydroxytryptophan was dissolved in a small volume of concentrated HCl and then further diluted by distilled water and back titrated to pH 5 by the addition of NaOH. All drugs were administered 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.

2.2. Measurement of emesis, head-twitch and ear-scratch responses

Depending upon the agent used to induce emesis in shrews, immediately following the injection of the emetogen, each shrew was placed in the observation cage and the frequency of vomiting (oral ejections of food or liquid; mean ± S.E.M.) was recorded for each individual shrew for the next 30 or 60 min. The head-twitch response in shrews is a behaviour (Darmani et al., 1994) similar to that observed in mice (Darmani, 2001d) and is analogous to wet-dog shakes in rats (Darmani and Ahmad, 1999). It is a distinctive behaviour 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 response frequency was scored by a multiple tally counter by a trained observer, and depending upon the head-twitch response inducer, the behaviour was recorded for either 30 or 60 min following administration of the serotonergic agent. The ear-scratch response was unexpectedly produced by 2-methylserotonin in shrews, which is a rapid scratching movement of the head, neck or lateral area by either hindlimb. The frequency of ear-scratch episodes was also scored by a tally counter (Darmani et al., 1994). An ear-scratch episode produced by a particular hindlimb consisted of one or more repetitive scratches with less than 2 s in between. If the interval between consecutive scratches by a particular hindlimb was greater than 2 s, the scratches were considered as separate episodes. If scratches were produced by alternative hindlegs, then each scratch was considered as a separate episode.

2.3. Head-twitch response and emesis studies

Preliminary studies in this laboratory have shown that intraperitoneal administration of 5-HTP produces both vomiting and the head-twitch response in shrews in a dose-dependent manner (Darmani, 1998; Zhao, 1996). To investigate the optimum dose at which the peripheral amino acid decarboxylase inhibitor carbidopa (0, 10, 20 and 40 mg/kg, $n = 9-12$ shrews per group) affects the production of 5-HTP-induced head-twitch response and emesis in shrews, two doses of 5-HTP were utilized: a 50 mg/kg dose at which 5-HTP by itself produces no behaviour and a 100 mg/kg dose which causes robust frequencies of vomiting and head-twitch responses in the least shrew. The present protocols were based upon our previous emesis (Darmani, 1998, 2001a,b,c) and head-twitch response (Darmani et al., 1994) studies. On the test day, shrews 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 × 18 × 21 cm clean clear plastic cage 30 min prior to experimentation. To evaluate whether pretreatment with carbidopa may alter the frequencies of 5-HTP-induced emesis and head-twitch response, at 0 min different groups of shrews were injected intraperitoneally (i.p.) with either vehicle or the cited doses of carbidopa. Immediately following injection, each shrew was placed back in the observation cage and offered four meal worms (*Tenebrio* sp.) to eat. At 30 min, the treated shrews received either a 50 or a 100 mg/kg (i.p.) dose of 5-HTP and the frequencies of induced vomiting and head-twitch response were recorded for each individual shrew for the next 60 min as described above.

To demonstrate whether delta-9-THC produces differential inhibitory effects when emesis produced by 5-HTP via central (i.e. in the presence of carbidopa) and peripheral/central (i.e. in the absence of carbidopa) mechanisms, different large groups of shrews were injected i.p. with either vehicle (i.e. control group), a submaximal (10 mg/kg) or a maximal (20 mg/kg) dose of carbidopa at 0 min and were then offered four meal worms. At 20 min, each subgroup of carbidopa-treated shrews received varying doses of delta-9-THC (0, 1, 2.5, 5,10 or 20 mg/kg, i.p., $n = 8-10$ shrews per group). At 30 min, each shrew was treated with 5-HTP (100 mg/kg, i.p.) and then observed individually for the next 60 min. The frequency of emesis and head-twitch response were recorded as described above. A 10 mg/kg dose of delta-9-THC nearly completely prevented the ability of 5-HTP (100 mg/kg in the presence of 20 mg/kg carbidopa) to induce both the head-twitch response and emesis via central mechanisms. To demonstrate whether both inhibitory effects are mediated via cannabinoid CB_1 receptors, the ability of the selective cannabinoid CB_1 receptor antagonist to reverse the inhibitory actions of delta-9-THC was investigated. Thus, at 0 min a large group of shrews received an i.p. dose of carbidopa (20 mg/kg), while subgroups of which received a subcutaneous (s.c.) injection of a dose of SR 141716A (0, 1, 5 or 10 mg/kg, $n = 8-9$ per group). Each shrew was treated with a 10 mg/kg dose of delta-9-THC (i.p.) at 20 min. At 30 min, each shrew received a 100 mg/kg (i.p.) dose of 5-HTP and observed for

the next 60 min. The frequencies of induced head-twitch responses and vomits were recorded during the following 60 min as described previously.

In the final series of experiments the ability of delta-9-THC to prevent emesis produced by systemically administered, peripherally acting nonselective (serotonin) and central + peripheral acting selective (2-methylserotonin) 5-HT_3 receptor agonists, which directly stimulate serotonin 5-HT_3 receptors to induce emesis was investigated. Thus, at 0 min different groups of shrews received varying doses of delta-9-THC (0, 1, 5, 10 or 20 mg/kg, i.p, $n = 8–10$ shrews per group) and 10 min later an i.p. dose of either serotonin (5 mg/kg) or 2-methylserotonin (5 mg/kg). The frequency of induced vomiting was recorded for the next 30 min as described earlier. 2-Methylserotonin unexpectedly produced both head-twitch response and the ear-scratch response which were extremely sensitive to the inhibitory effects of delta-9-THC. Thus, the effect of lower doses of the cannabinoid (0.025, 0.1 and 0.25 mg/kg) were also investigated on these behaviors.

2.4. Statistical analysis

A p value less than 0.05 was necessary to achieve statistical significance. Two-factor analysis of the frequency of head-twitch response and of emesis data was performed using Poisson regression and post hoc analysis by chi-square tests of the regression parameters. One-factor analysis of the frequency of head-twitch and ear-scratch responses and emesis data was completed using the Kruskal–Wallis test and post hoc analysis by Dunn's multiple comparison procedure. Two-factor analysis of the incidence of emesis was performed using logistic regression and post hoc analysis by chi-square tests of the regression parameters; one-factor analysis was completed using Fisher's exact test with post hoc analysis by the same method. All the preceding analyses were performed using SAS® statistical software (SAS Institute, Cary, NC). The ID_{50} values (the inhibitory dose that prevented emesis in 50% of shrews, or the dose which reduced the frequency of a behavior by 50%) were calculated by the use of a computerized program (GraphPad InPlot, San Diego, CA) and the groups compared using two-sample t-tests.

3. Results

3.1. Effects of carbidopa on 5-HTP-induced head-twitch response and vomiting

Poisson regression of head-twitch response for two-factor analysis of carbidopa and 5-HTP doses resulted in highly significant differences between both carbidopa ($\chi^2(3) = 2805$, $p < 0.0001$) and 5-HTP ($\chi^2(1) = 1460$, $p < 0.0001$) treatments (Fig. 1A). There was also a significant interaction between carbidopa and 5-HTP treatments ($\chi^2(3) = 235$, $p < 0.0001$). Post hoc chi-square tests showed

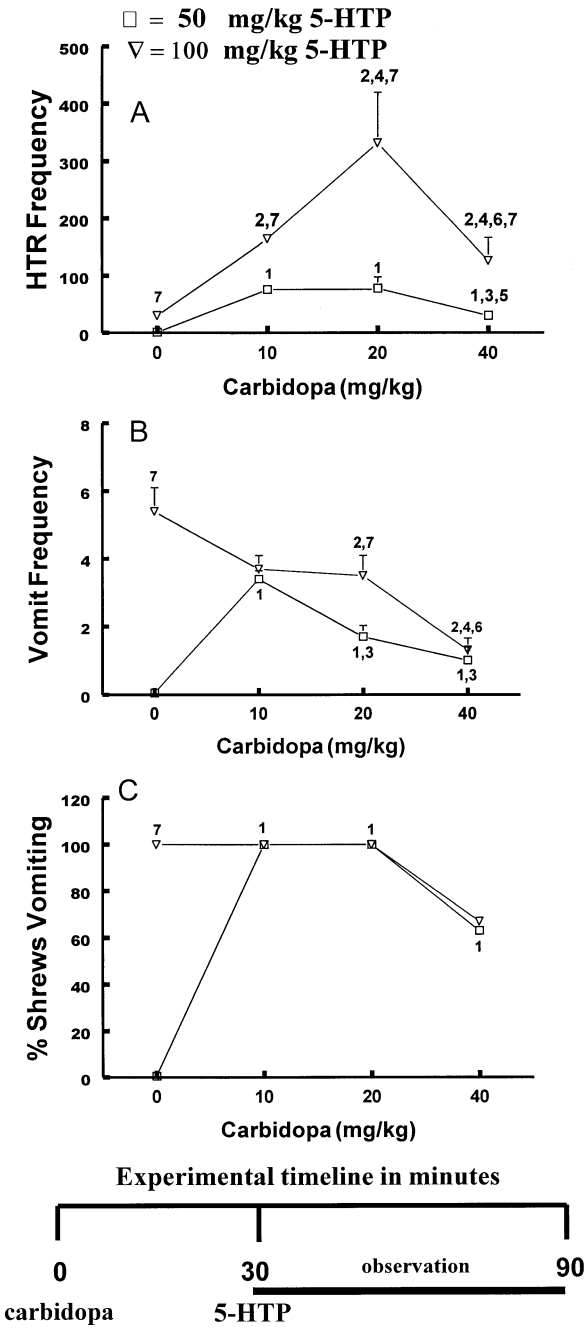

Fig. 1. Dose–response effects of the cited doses of carbidopa in the least shrew on the 5-HTP-induced (□ = 50 mg/kg and ▽ = 100 mg/kg): (A) head-twitch response (HTR) (mean ± S.E.M.), (B) vomit frequency (mean ± S.E.M.) and (C) percent shrews vomiting. 1 = significantly different from 0 mg/kg carbidopa in 50 mg/kg 5-HTP-treated group. 2 = significantly different from 0 mg/kg carbidopa in 100 mg/kg 5-HTP-treated group. 3 = significantly different from 10 mg/kg carbidopa in 50 mg/kg 5-HTP-treated group. 4 = significantly different from 10 mg/kg carbidopa in 100 mg/kg 5-HTP-treated group. 5 = significantly different from 20 mg/kg carbidopa in 50 mg/kg 5-HTP-treated group. 6 = significantly different from 20 mg/kg carbidopa in 100 mg/kg 5-HTP-treated group. 7 = significantly different from 50 mg/kg 5-HTP in carbidopa dose.

that the 50 mg/kg dose of 5-HTP did not produce a significant number of head-twitches. However, in the presence of 10, 20 and 40 mg/kg doses of carbidopa, the 50 mg/

kg 5-HTP dose produced robust but bell-shaped increases in the mean frequency of head-twitch response (all at $p < 0.0001$ relative to carbidopa vehicle-treated control group). On the other hand, the 100 mg/kg 5-HTP produced 30 ± 6 head-twitches by itself which was significantly enhanced in a bell-shaped dose–response manner by the cited doses of carbidopa (all at $p < 0.0001$). Furthermore, relative to its 50 mg/kg dose, 5-HTP at 100 mg/kg caused greater increases in head-twitch response frequency across the cited doses of carbidopa ($p < 0.0001$ in each case).

Poisson regression for two-factor analysis of vomit frequency for carbidopa and 5-HTP treatments resulted in highly significant differences between various doses of both carbidopa ($\chi^2(3) = 36.4$, $p < 0.0001$) and 5-HTP ($\chi^2(1) = 35.1$, $p < 0.0001$) (Fig. 1B). There was also a significant interaction between carbidopa and 5-HTP treatment groups ($\chi^2(3) = 42.04$, $p < 0.0001$). Post hoc chi-square tests indicated that the 50 mg/kg dose of 5-HTP by itself failed to induce significant emesis. Carbidopa at 10 mg/kg significantly potentiated the ability of 5-HTP (50 mg/kg) to induce vomiting (3.4 ± 0.2, $p < 0.0001$). However, larger doses of carbidopa (20 and 40 mg/kg) significantly and dose-dependently reduced the latter vomiting frequency (1.7 ± 0.3 and 1 ± 0.4, respectively, both at $p < 0.0001$). On the other hand, the 100 mg/kg dose of 5-HTP caused a significant number of emesis by itself (5.4 ± 0.7) which was dose-dependently reduced by carbidopa (3.7 ± 0.4 ($p > 0.05$), 3.5 ± 0.6 ($p < 0.05$) and 1.34 ± 0.4 ($p < 0.0001$), respectively). In addition, the 100 mg/kg dose of 5-HTP produced a greater number of vomits relative to its 50 mg/kg dose in the 0 ($p < 0.001$) and 20 ($p < 0.02$) mg/kg carbidopa treatment groups. The logistic regression analysis of two factors for the ability of carbidopa (0, 10, 20 and 40 mg/kg) to promote 5-HTP (50 and 100 mg/kg)-induced emesis resulted in significant differences among carbidopa treatments ($\chi^2(3)$ $p < 0.04$) but not between 5-HTP doses (Fig. 1C). Post hoc chi-square test indicated that in the 50 mg/kg 5-HTP dose, increasing doses of carbidopa (10, 20 and 40 mg/kg) significantly ($p < 0.006$, $p < 0.006$, and $p < 0.04$, respectively) increased the % of shrews vomiting. Although there was no main effect among 5-HTP doses, post hoc analysis revealed a significant ($p < 0.006$) difference between the 50 and 100 mg/kg doses of 5-HTP at 0 mg/kg carbidopa dose.

3.2. Effects of delta-9-THC on 5-HTP (100 mg/kg)-induced head-twitch response and vomiting in the absence and presence of carbidopa

Poisson regression for two-factor analysis of head-twitch response for delta-9-THC (0, 1, 2.5, 5 and 10 mg/kg) and carbidopa (0, 10 and 20 mg/kg) treatments resulted in highly significant differences among both delta-9-THC ($\chi^2(4) = 21918$, $p < 0.0001$) and carbidopa ($\chi^2(2) = 3648$, $p < 0.0001$) treatments (Fig. 2A). There was also a significant interaction between carbidopa and delta-9-THC treatments ($\chi^2(8) = 277$, $p < 0.0001$). Carbidopa pretreatment (10 and 20 mg/kg)

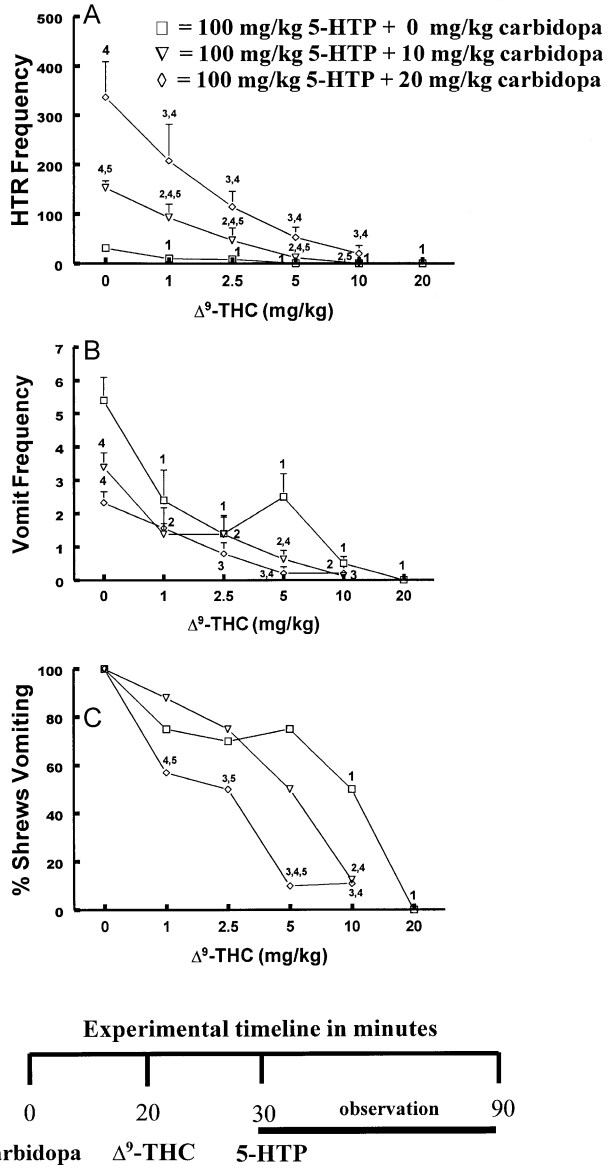

Fig. 2. Inhibitory dose–response effects of the cited doses of delta-9-THC on the ability of 100 mg/kg 5-HTP (i.p.) in the least shrew to produce: (A) head-twitch response (HTR) (mean ± S.E.M.), (B) vomit bouts (mean ± S.E.M.) and (C) emesis in the number of shrews tested in the absence (□) and presence of 10 mg/kg (▽) and 20 mg/kg (◇) carbidopa. 1 = significantly different from 0 mg/kg delta-9-THC in 0 mg/kg carbidopa-treated group. 2 = significantly different from 0 mg/kg delta-9-THC in 10 mg/kg carbidopa-treated group. 3 = significantly different from 0 mg/kg delta-9-THC in 20 mg/kg carbidopa-treated group. 4 = significantly different from 0 mg/kg carbidopa in delta-9-THC dose. 5 = significantly different from 20 mg/kg carbidopa in delta-9-THC dose.

significantly and dose-dependently increased (394% and 987% relative to a 0 mg/kg carbidopa group, $p < 0.0001$ for both cases) the 5-HTP-induced head-twitch response in the shrew control group which had received delta-9-THC vehicle. In the absence of carbidopa, the cited doses of delta-9-THC dose-dependently and significantly ($p < 0.0001$ in each case) attenuated 5-HTP-induced head-twitch response by 68%, 71%, 98% and 100% relative to its vehicle-treated

control group. Since the effect of a 20 mg/kg dose of delta-9-THC was also investigated in this treatment group, these results were also analyzed by the Kruskal–Wallis one-way analysis of variance (ANOVA) test ($\chi^2(5)=41.2$, $p<0.0001$). Post hoc tests indicated that the 20 mg/kg delta-9-THC dose also significantly attenuated ($p<0.0001$) 5-HTP-induced head-twitch response by 100%. In the presence of carbidopa these doses of delta-9-THC also significantly reduced the frequency of 5-HTP-induced head-twitch response by 39%, 69%, 92% and 100% in the 10 mg/kg carbidopa group; and by 38%, 66%, 84% and 94% in the 20 mg/kg carbidopa-treated shrews ($p<0.0001$ for each case). Irrespective of carbidopa dose level, it appears that the degree of inhibition of head-twitch response by delta-9-THC is consistent across carbidopa doses since ID_{50} values for the inhibition of 5-HTP-induced head-twitch response in the 0 (0.63 ± 0.4), 10 (1.7 ± 1.23) and 20 (1.84 ± 1.1) mg/kg carbidopa treatment groups are not significantly different from each other. Although the inhibitory ID_{50s} of delta-9-THC against 5-HTP-induced head-twitch response in different carbidopa treatment groups were similar, the mean head-twitch response frequency both in the 10 and 20 mg/kg carbidopa-exposed groups across the cited doses of delta-9-THC were generally greater ($p<0.0001$) than their corresponding head-twitch responses in the absence of carbidopa. The head-twitch response frequency in the 20 mg/kg carbidopa treatment group was also significantly greater across each corresponding dose of delta-9-THC in the 10 mg/kg carbidopa-treated group ($p<0.0001$ for each case).

Poisson regression for two-factor analysis of reductions in the frequency of 5-HTP (100 mg/kg)-induced vomits by delta-9-THC (0, 1, 2.5, 5 and 10 mg/kg) in different carbidopa treatment groups (0, 10 and 20 mg/kg) resulted in highly significant differences both among the cited doses of delta-9-THC ($\chi^2(4)=101$, $p<0.0001$) and carbidopa ($\chi^2(2)=18.4$, $p<0.0001$) (Fig. 2B). In the absence of carbidopa, post hoc analysis revealed that relative to its vehicle-treated control group, delta-9-THC dose-dependently and significantly reduced the frequency of 5-HTP-induced vomits by 56% ($p<0.002$), 74% ($p<0.0001$), 54% ($p<0.003$) and 91% ($p<0.0001$), respectively. Since this treatment group also contained an extra 20 mg/kg delta-9-THC exposure subgroup, the Kruskal–Wallis ANOVA test followed by post hoc analysis were performed ($\chi^2(5)=28.1$, $p<0.0001$) which indicated that the 20 mg/kg delta-9-THC significantly and completely blocked the ability of 5-HTP to produce vomiting ($p<0.0001$). In the presence of carbidopa, the different doses of delta-9-THC also significantly reduced the frequency of 5-HTP-induced vomits by 59% ($p<0.0001$), 59% ($p<0.009$), 82% ($p<0.0006$) and 96% ($p<0.001$) in the 10 mg/kg carbidopa exposure group; and by 30% ($p>0.05$), 65% ($p<0.01$), 91% ($p<0.0009$) and 91% ($p<0.002$) in the 20 mg/kg carbidopa treatment group. In addition, in the 0 mg/kg delta-9-THC-exposed group, the 10 and 20 mg/kg carbidopa treatment groups respectively exhibited 37% ($p<0.04$) and 57% ($p<0.001$) less vomits relative to its 0 mg/kg control group. Across the various delta-9-THC treatments, only for the 5 mg/kg delta-9-THC group such respective differences across the 10 (76%, $p<0.01$) and 20 mg/kg (92%, $p<0.0007$) carbidopa treatments were observed relative to the 0 mg/kg carbidopa control group. In addition, no significant difference was observed between the 10 and 20 mg/kg carbidopa exposure groups across the cited doses of delta-9-THC. Moreover, delta-9-THC attenuated the frequency of 5-HTP-induced vomiting with similar ID_{50} values (0.96 ± 2.4, 1.06 ± 1.96 and 2.1 ± 1.55, respectively, $p>0.05$) across the 0, 10 and 20 mg/kg carbidopa exposure groups.

The logistic regression for a two-factor analysis of the percentage of shrews being protected from 5-HTP (100 mg/kg)-induced emesis by delta-9-THC (0, 1, 2.5, 5 and 10 mg/kg) in different carbidopa exposure groups (0, 10 and 20 mg/kg) resulted in highly significant differences among the cited doses of delta-9-THC ($\chi^2(4)=22.2$, $p<0.0002$) but not between the various doses of carbidopa ($p=0.06$) (Fig. 2C). Post hoc tests revealed that delta-9-THC up to 10 mg/kg did not significantly protect shrews from vomiting in the 0 mg/kg carbidopa treatment group (Fig. 2C). As in this treatment group the inhibitory effect of a 20 mg/kg dose of delta-9-THC was also investigated, the results of this portion of the experiment were further analyzed via the Fisher's exact test ($\chi^2(5)=20.6$, $p<0.0003$). Post hoc tests indicated that the 10 and 20 mg/kg doses of delta-9-THC significantly protected shrews by 50% ($p<0.03$) and 100% ($p<0.0001$) from vomiting. In the 10 mg/kg carbidopa-exposed shrews, delta-9-THC (1, 2.5, 5 and 10 mg/kg) protected shrews from the 5-HTP-induced emesis by 11%, 25%, 50% and 97%, respectively. However, a significant effect was only observed at its 10 mg/kg tested dose ($p<0.008$). In the 20 mg/kg carbidopa exposure group, delta-9-THC more potently protected shrews from vomiting since significant protection (50%, 90% and 89%, respectively) occurred at its 2.5 ($p<0.05$), 5 ($p<0.005$) and 10 mg/kg ($p<0.006$) doses. In addition, although the main effect ($p=0.06$) was just outside 0.05 for the effect of different doses of carbidopa in the two-factor analysis, post hoc tests indicated significant differences between 0 and 20 mg/kg, and between 10 and 20 mg/kg carbidopa doses in the 5 mg/kg delta-9-THC dose. In addition, two-tailed *t*-test indicated that the delta-9-THC ID_{50} in protecting shrews from vomiting in the 20 mg/kg carbidopa exposure group (1.92 ± 1.3 mg/kg) is significantly lower ($p<0.05$ and $p<0.0003$, respectively) than both its corresponding values across the 0 (10 ± 1.1 mg/kg) and 10 mg/kg (6 ± 1.5 mg/kg) carbidopa treatment groups.

3.3. Reversal of the inhibitory effects of delta-9-THC on 5-HTP-induced head-twitch response and emesis

The canabinnoid CB_1 receptor antagonist SR 141716A (1, 5 and 10 mg/kg) dose-dependently reversed the ability of a 10 mg/kg dose of delta-9-THC to completely block the

capacity of 5-HTP to produce the head-twitch response ($\chi^2(3) = 19.79$, $p < 0.002$) (Fig. 3A). Post hoc analysis revealed that significant reversals ($p < 0.01$ for each case) occurred from its lowest tested dose. Likewise, SR 141716A increased in a dose-dependent manner the frequency of vomits by reversing the antiemetic effect of delta-9-THC

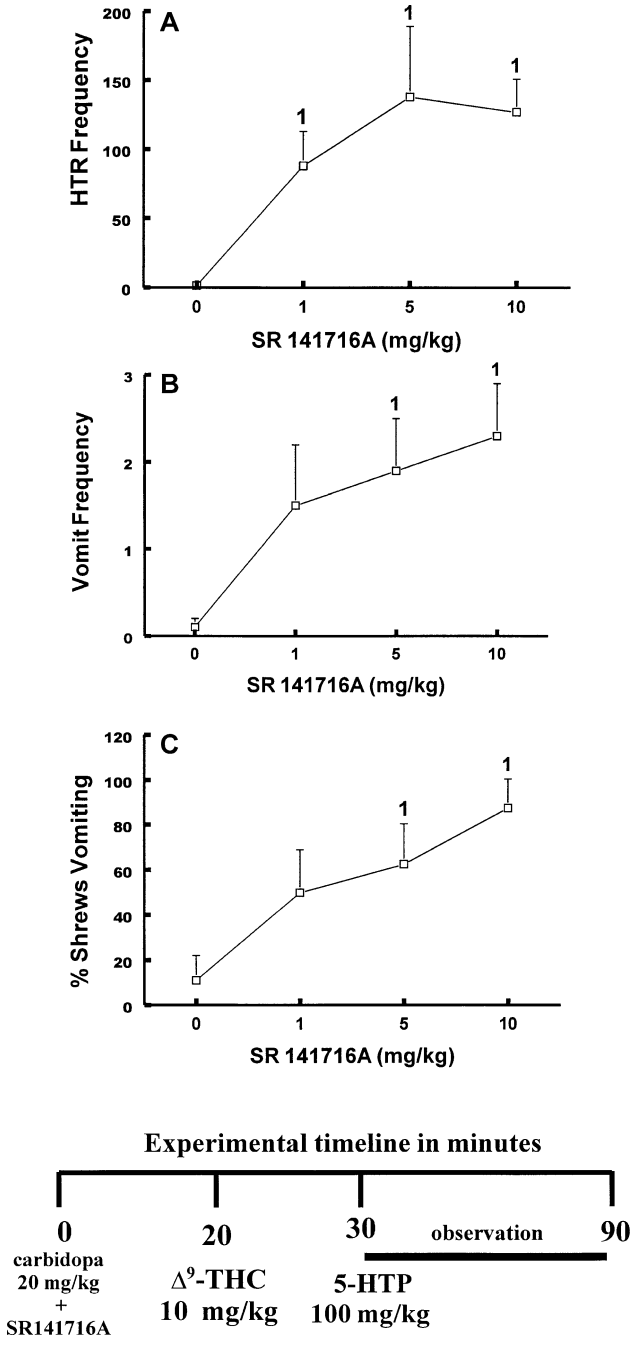

Fig. 3. The ability of the cited doses of the cannabinoid CB_1 receptor antagonist SR 141716A to reverse the complete inhibitory effects of a 10 mg/kg dose of delta-9-THC on the capacity of a 100 mg/kg dose of 5-HTP to produce: (A) the head-twitch response (HTR) (mean ± S.E.M.), (B) vomit bouts (mean ± S.E.M.) and (C) emesis in the number of shrews tested in the presence of a 20 mg/kg dose of carbidopa. 1 = significantly different from corresponding 0 mg/kg SR 141716A-treated controls.

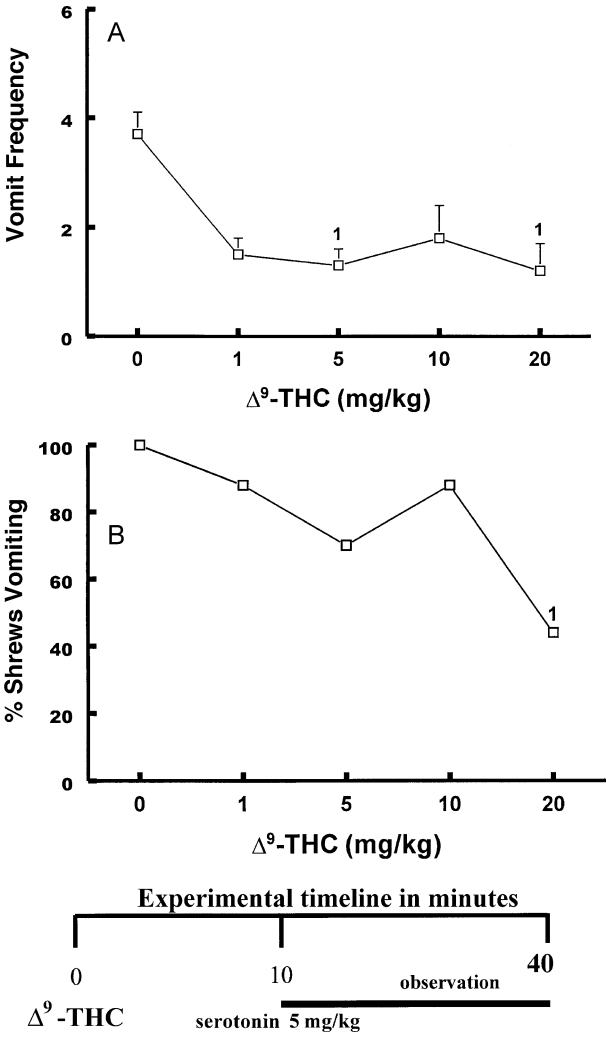

Fig. 4. The dose–response effects of delta-9-THC on serotonin (5 mg/kg, i.p.)-induced emesis in the least shrew. Graph A depicts attenuations in the mean frequency (± S.E.M.) of induced vomiting, whereas graph B shows reductions in percentage of shrews vomiting. 1 = significantly different from 0 mg/kg delta-9-THC control group.

against 5-HTP-induced emesis ($\chi^2(3) = 9.5$, $p < 0.02$) (Fig. 3B). However, significant reversals occurred at its 5 ($p < 0.03$) and 10 mg/kg ($p < 0.006$) doses. Fisher's exact test showed that the ability delta-9-THC to protect shrews from vomiting was dose-dependently decreased by the cited doses of SR 141716A ($\chi^2(3) = 10.4$, $p < 0.0003$) (Fig. 3C). Significant reductions ($p < 0.05$ and $p < 0.003$, respectively) in emesis protection were seen at its 5 and 10 mg/kg doses.

3.4. The effects of delta-9-THC on behaviours produced by direct acting nonselective and "selective" serotonin 5-HT$_3$ receptor agonists

Delta-9-THC (1, 5, 10 and 20 mg/kg) reduced the frequency of vomiting induced by a 5 mg/kg dose of serotonin by 59%, 65%, 53% and 68% (KW(4) = 13.82, $p < 0.008$) (Fig. 4A). However, significant reductions oc-

curred at its 5 ($p<0.05$) and 20 mg/kg ($p<0.05$) doses only. Delta-9-THC also reduced the percentage of shrews vomiting (13%, 30%, 13% and 56%, respectively) ($\chi^2(4)=13.82$, $p<0.05$) (Fig. 4B). However, a significant effect was only seen at its 20 mg/kg dose ($p<0.03$). Likewise, delta-9-THC (0.025, 0.1, 0.25, 1, 5, 10 and 20 mg/kg) reduced the frequency of emesis produced by a 5 mg/kg dose of the "selective" serotonin 5-HT_3 receptor agonist 2-methylserotonin (KW(7)=21.1, $p<0.004$) (Fig. 5A). However, a significant reduction (79% relative to vehicle control, $p<0.05$) was only observed at the highest tested dose of delta-9-THC. The Fisher's exact test also indicated that only the 20 mg/kg dose of delta-9-THC significantly reduced (75%) the number of shrews vomiting in response to 2-methyl-5-HT injection ($\chi^2(7)=26.1$, $p<0.0003$) (Fig. 5B). Unexpectedly, and unlike serotonin, 2-methylserotonin also

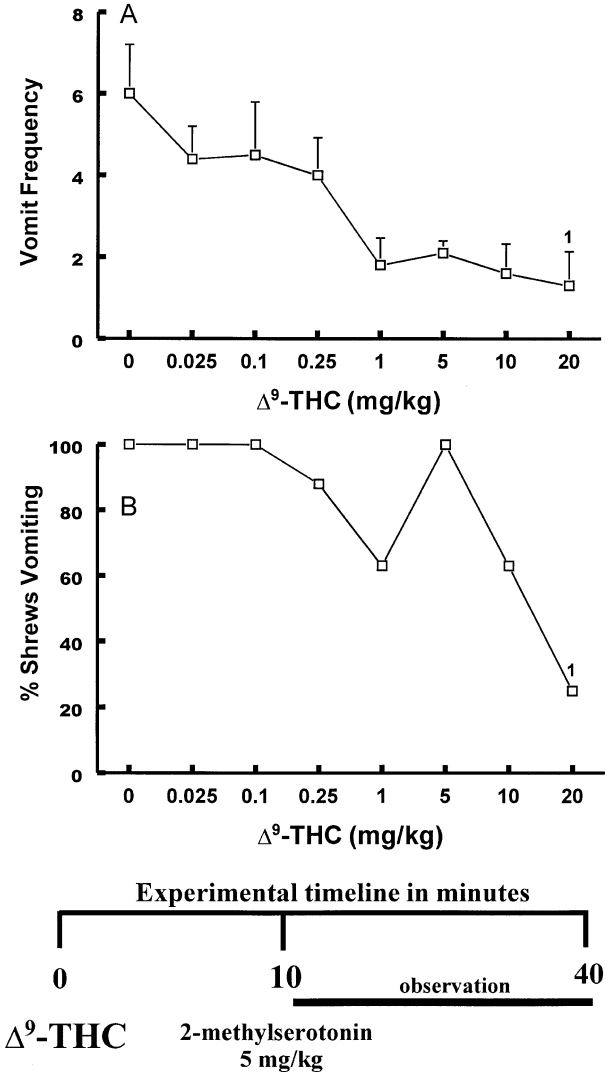

Fig. 5. The dose–response effects of delta-9-THC on 2-methylserotonin (5 mg/kg, i.p.)-induced vomiting in the least shrew. Graph A shows attenuations in the mean frequency (± S.E.M.) of induced emesis, whereas graph B depicts reductions in percentage of shrews vomiting. 1 = significantly different from 0 mg/kg delta-9-THC control group.

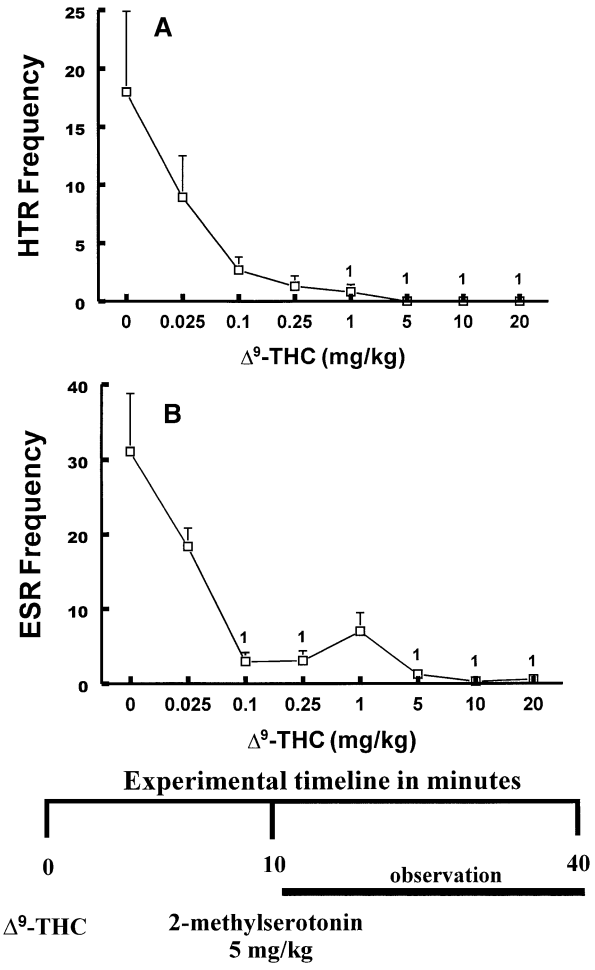

Fig. 6. The inhibitory dose–response effects of delta-9-THC on 2-methylserotonin (5 mg/kg, i.p.)-induced head-twitch response (HTR) and ear-scratch response (ESR) in the least shrew. Graphs A and B show reductions in the respective mean frequencies (± S.E.M.) of HTR and ESR caused by delta-9-THC. 1 = significantly different from 0 mg/kg delta-9-THC control group.

produced the head-twitch response and the ear-scratch response in shrews. Both of these induced behaviors were sensitive to the inhibitory effects of delta-9-THC (KW(7)=37.6, $p<0.0001$, and KW(7)=43.6, $p<0.0001$, respectively) (Fig. 6). The head-twitch response frequency was reduced by 51%, 84%, 92%, 96%, 100%, 100% and 100%, respectively, by the cited doses of delta-9-THC and significant effects were seen from 1 mg/kg and above ($p<0.05$ for every case) (Fig. 6A). The ear-scratch response frequency was reduced by 42% ($p>0.05$), 90% ($p<0.05$), 90% ($p<0.05$), 72% ($p>0.05$), 96% ($p<0.01$), 99% ($p<0.001$) and 98% ($p<0.001$) (Fig. 6B).

4. Discussion

The overall findings of the present diverse behavioral investigations suggest that activation of cannabinoid CB_1 receptors by delta-9-THC attenuates emesis produced by the

serotonin precursor, 5-HTP, probably via both central and peripheral as well as pre- and post-synaptic mechanisms. These conclusions are based on the following.

4.1. Establishment of peripherally optimal effective dose of carbidopa

The initial carbidopa plus 5-HTP experiments were necessary to establish the optimum protocols for the investigation of the inhibitory effects of delta-9-THC on 5-HTP-evoked, centrally mediated serotonin 5-HT_{2A} receptor-induced head-twitch response, and peripheral plus centrally mediated serotonin 5-HT_3-receptor induced emesis, in the least shrew (see introduction). Preliminary experiments showed that carbidopa at the doses employed in this study did not produce either the head-twitch response or emesis by itself. Although the dose–response effects of carbidopa pretreatment on 5-HTP-induced head-twitch response has already been published in mice (Handley and Singh, 1986), similar studies on 5-HTP-induced emesis have not yet been described. Indeed, the latter authors have shown that 10–15 mg/kg doses of carbidopa optimally potentiate the ability of 5-HTP (180 mg/kg) to induce the head-twitch response in a dose-dependent but bell-shaped manner. To account for the bell-shaped dose–response effect, it was suggested that high doses of carbidopa can pass the blood–brain barrier and inhibit the conversion of 5-HTP to 5-HT in the CNS. Likewise, in this study, we show that carbidopa (10–40 mg/kg) also potentiated the ability of two doses of 5-HTP (50 and 100 mg/kg) to induce the head-twitch response in a bell-shaped manner in an insectivore species, the least shrew. Depending on the dose of 5-HTP utilized, the optimal dose of carbidopa required to maximally potentiate the induced head-twitch response varied between 10 and 20 mg/kg, whereas a large dose (40 mg/kg) attenuated the attained maximal head-twitch response frequency. In addition, this study further shows that carbidopa not only increases the capacity of an effective systemic dose of 5-HTP (100 mg/kg) which can induce the head-twitch response by itself, but also allows an ineffective dose of 5-HTP (50 mg/kg) which fails to induce head-twitch response when administered alone to produce the behavior. These findings appear to confirm in shrews the published conclusions that blockade of conversion of 5-HTP to 5-HT in the periphery by an effective dose of carbidopa potentiates the ability of 5-HTP to increase 5-HT synthesis and turnover in the CNS leading to the observed increase in frequency of head-twitch response in rodents (Endo, 1985; Goodrich et al., 1989; Handley and Singh, 1986; Gartside et al., 1992). The observed bell-shaped dose–response in 5-HTP-induced head-twitch response could also be due to the discussed enhanced serotonin levels in the CNS which would simultaneously stimulate the inhibitory somatodendritic 5-HT_{1A} autoreceptors found on the raphe cell bodies and subsequently decrease both 5-HT release (Gartside et al., 1992) and the induced head-twitch response (Darmani and Reeves, 1996). However, this notion seems to be unlikely in the least shrew since the selective 5-$HT_{2A/C}$ receptor agonist, DOI, also produces the head-twitch response in a bell-shaped dose–response manner (Darmani et al., 1994).

Unlike the observed potentiation of the head-twitch response, significant blockade of peripheral conversion of 5-HTP to 5-HT by carbidopa at doses well below 40 mg/kg reduced the frequency of both doses of 5-HTP to induce vomits in a dose-dependent fashion. These results further show that following systemic administration of 5-HTP, the newly synthesized serotonin in the periphery is rapidly metabolized since unlike its 100 mg/kg dose, 5-HTP at 50 mg/kg failed to induce emesis by itself. Moreover, the lowest tested dose of carbidopa potentiated the ability of the 50 mg/kg dose of 5-HTP to induce vomiting while reducing the vomiting frequency of its 100 mg/kg dose. The concomitant but opposing respective stimulatory and inhibitory effects of carbidopa on the frequencies of 5-HTP-induced head-twitch response and emesis implies that: (1) in the presence of an adequate dose of carbidopa (10–20 mg/kg), the induced emesis is due to conversion of 5-HTP to 5-HT in the CNS, and (2) following systemic administration, larger doses of 5-HTP are required to induce emesis via a peripheral mechanism (i.e. in the absence of carbidopa) since a nonemetic dose of 5-HTP (i.e. 50 mg/kg) can induce vomiting in the presence of a relatively small dose of carbidopa. The latter notion is in agreement with previously published findings, that although the peripherally acting quaternary analogue of serotonin, 5-HTQ [trimethylserotonin iodide, a selective 5-HT_3 receptor agonist (Dukat et al., 1991)], has 18 times greater affinity for the 5-HT_3 receptor site, it is a significantly less potent emetogen than the central/peripheral-acting 5-HT_3 receptor agonist, 2-methylserotonin (Darmani, 1998). A role for the peripheral component of indirect emetic action of 5-HTP is further supported by the observations that systemically administered 5-HT or 5-HTQ produce emesis and neither of which can penetrate the blood–brain barrier (Darmani, 1998; present investigation). Furthermore, as discussed in the introduction, selective 5-HT_3 receptor antagonists prevent cisplatin-induced vomiting via blockade of both central and peripheral serotonin 5-HT_3 receptors. Thus, it can be concluded that 5-HTP indirectly induces emesis via both central and peripheral mechanisms. However, the role of serotonin in the production of emesis seems to be complicated since several serotonergic receptors appear to differentially modulate vomiting activity. Although 5-HT_3 receptor is the predominent serotonin emetic receptor, activation of 5-HT_4 receptor may also contribute to production of emesis (Horikoshi et al., 2001; Veyrat-Follet et al., 1997). In addition, selective 5-HT_{1A} [e.g. 8-OH-DPAT, (±)-8-hydroxy-dipropylaminotetralin HBr] and 5-$HT_{2A/C}$ [e.g. DOI; (±)-2,5-dimethoxy-4-iodoamphetamne hydrochloride] receptor agonists at doses 1-3 mg/kg seem to prevent vomiting produced by some emetic stimuli including cisplatin (Okada et al., 1994, 1995). Thus, simultaneous

activation of these serotonin receptor subtypes by serotonin derived from peripherally administered 5-HTP may complicate the interpretation of the present data. However, both 8-OH-DPAT and DOI lack antiemetic activity against cisplatin-induced emesis in the least shrew (Darmani, unpublished observations). Moreover, the least shrew is very sensitive to DOI which induces the head-twitch response in this species in a bell-shaped manner with its maximal effect occurring between 0.31 and 0.63 mg/kg (Darmani et al., 1994). On the other hand, unlike rats, the least shrew is not very sensitive to 5-HT_{1A} agonists since only large doses of 8-OH-DPAT (>2.5 mg/kg) can induce the 5-HT_{1A} receptor mediated serotonin syndrome in this species (Darmani and Zhao, 1998). Thus, simultaneous activation of serotonin 5-HT_{1A} and 5-$HT_{2A/C}$ receptors do not seem to play a major role in the modulation of the 5-HTP-induced emesis in this animal model of emesis.

4.2. Delta-9-THC prevents serotonergically mediated head-twitch response and emesis behaviours

Delta-9-THC pretreatment dose-dependently attenuated the frequency of a behaviorally effective dose of 5-HTP (100 mg/kg) to induce the head-twitch response both in the absence and presence of carbidopa (10 or 20 mg/kg) with similar ID_{50} values. Thus, although carbidopa potentiates the ability of 5-HTP to induce the head-twitch response, it does not alter the inhibitory capacity of delta-9-THC to attenuate this centrally mediated behavior. Delta-9-THC also dose-dependently attenuated the frequency of 5-HTP-induced emesis in the absence and presence of carbidopa with similar ID_{50} potencies. However, at the 5 mg/kg dose of delta-9-THC, 5-HTP induced significantly less vomits in the 10 and 20 mg/kg carbidopa-exposed shrews relative to the 0 mg/kg control group. This finding indicates that delta-9-THC may more potently prevent vomiting when emesis is induced by 5-HTP via a central mechanism (i.e. in the presence of carbidopa). This notion is further supported by the differential ability of delta-9-THC to protect shrews from 5-HTP-induced vomiting in the presence and absence of carbidopa. Indeed, the current results show that delta-9-THC more effectively and dose-dependently protected shrews from vomiting in the presence of a 20 mg/kg dose of carbidopa ($ID_{50} = 1.92 \pm 1.3$ mg/kg) relative to its absence ($ID_{50} = 10 \pm 1.1$ mg/kg, $p < 0.0003$) or in the presence of a 10 mg/kg dose of the peripheral decarboxylase inhibitor ($ID_{50} = 6 \pm 1.5$ mg/kg, $p < 0.05$). Furthermore, only the highest tested dose of delta-9-THC significantly blocked the different parameters of emesis produced by the direct acting nonselective (5-HT) and the "selective" (2-methylserotonin) serotonin 5-HT_3 receptor agonists. Since 2-methylserotonin but not 5-HT following systemic administration can enter the CNS, the observed antiemetic effect of the large dose of delta-9-THC appears to be peripherally mediated. In addition, a closer inspection of the antiemetic dose–response effect of delta-9-THC against the centrally plus peripherally acting 5-HT agonists 2-methylserotonin and 5-HTP (in the absence of carbidopa) (Figs. 2B,C and 5A,B) shows 2 phases of inhibition. The initial inhibitory phase occurs at lower doses of delta-9-THC which is probably centrally mediated, while the second inhibition phase is observed at larger doses of delta-9-THC which is probably mediated by a peripheral mechanism. On the other hand, the antiemetic action of delta-9-THC against the peripherally acting (serotonin) or the centrally acting (5-HTP in the presence of carbidopa) serotonergic agents (Figs. 2B,C and 4A,B) mainly exhibits a one phase inhibition. The peripheral nature of the antiemetic effect of delta-9-THC is further supported from our unexpected observation that the "selective" 5-HT_3 receptor agonist 2-methylserotonin also produced two centrally mediated behaviours in shrews: (1) the head-twitch response and (2) the ear-scratch response both of which are 5-HT_{2A} receptor-mediated events (Darmani et al., 1994). These two behaviours were potently and completely blocked by low doses of delta-9-THC. Although 2-methylserotonin is generally considered selective for serotonin 5-HT_3 receptors, the present behavioral study and published binding data (Ismaiel et al., 1990) suggest it also binds serotonin 5-HT_{2A} receptors. Recent reviews on the gastrointestinal effects of cannabinoids have concluded that delta-9-THC-like agents act mainly, but not exclusively, via peripheral cannabinoid CB_1 receptors to reduce intestinal motility (Pertwee, 2001; Pinto et al., 2002) whereas central CB_1 receptors in the brainstem have been proposed to mediate the antiemetic action of cannabinoids (Van Sickle et al., 2001). Overall, the discussed findings support the notion that delta-9-THC prevents 5-HTP-induced vomiting via both central and peripheral mechanisms, with its peripheral antiemetic effects occurring at relatively larger doses.

One mechanism by which delta-9-THC may inhibit both 5-HTP-induced behaviours is via the presynaptic inhibition of release of the newly synthesized serotonin since structurally diverse cannabinoid agonists inhibit neurotransmitter release, including serotonin, via stimulation of presynaptic cannabinoid CB_1 receptors (Howlett et al., 2002; Schlicker and Kathman, 2001). Moreover, the cannabinoid CB_1 receptor antagonist SR 141716A, not only produces dose- and time-dependent increases in the levels and turnover of serotonin and dopamine in shrew forebrain and brainstem (Darmani et al., 2003b), but also induces vomiting, head-twitch and ear-scratch responses (Darmani et al., 2003b; Darmani and Pandya, 2000). In addition, the SR 141716A-induced behaviors were blocked by both structurally diverse cannabinoid CB_1/CB_2 receptor agonists and the serotonin 5-HT_{2A} receptor antagonist SR 46349B. Inhibition of 5-HTP-induced emesis and head-twitch response may also occur at the corresponding postsynaptic serotonergic 5-HT_3- and 5-HT_{2A}-receptors since their function can be modulated by cannabinoid CB_1/CB_2 receptor agonists. Indeed, cannabinoid receptors are co-expressed

with both serotonin 5-HT_{2A}- and 5-HT_3-receptors in some neurons in the CNS (Devlin and Christopoulos, 2002; Hermann et al., 2002), and inhibitory functional interactions have been reported between cannabinoid CB_1- and 5-HT_{2A}-receptors (Kimura et al., 1998; Cheer et al., 1999; Darmani, 2001d) as well as between cannabinoid CB_1- and 5-HT_3-receptors (Barann et al., 2002; Fan, 1995). Additionally, in the present and cited studies, structurally diverse cannabinoids reduced the ability of direct-acting selective: (1) serotonin 5-HT_{2A} receptor agonists to induce the postsynaptically mediated 5-HT_{2A} receptor-induced head-twitch response, and (2) 5-HT_3 agonists to produce emesis (present study) or currents through the serotonin 5-HT_3 receptor ligand-gated ion channels (Barann et al., 2002; Fan, 1995). Thus, pre- and postsynaptic mechanisms may account for the inhibitory actions of delta-9-THC on 5-HTP-induced behaviors.

4.3. Delta-9-THC prevents serotonergically induced head-twitch response and vomiting via cannabinoid CB_1 receptors

The antiemetic and head-twitch response reducing properties of delta-9-THC appear to be cannabinoid CB_1-receptor mediated events since the selective cannabinoid CB_1 receptor antagonist, SR 141716A, countered the inhibitory effects of an effective dose of delta-9-THC against both of these 5-HTP-induced behaviours. Furthermore, structurally diverse cannabinoid CB_1/CB_2 agonists attenuate cisplatin-induced (Darmani, 2001b,c; Darmani et al., 2003a) emesis as well as vomiting produced by other stimuli (Darmani, 2001a; Van Sickle et al., 2001) via cannabinoid CB_1 receptors in an ID_{50} order that is highly correlated with their: (I) affinity order for cannabinoid CB_1 receptors in the least shrew brain (Darmani et al., 2003a); (II) EC_{50} potency rank order for GTPγS stimulation in shrew brain (Darmani et al., 2003a); (III) ID_{50} order for reducing spontaneous locomotor activity and rearing behaviors (Janoyan et al., 2002); (IV) ID_{50} order for reducing the head-twitch and ear-scratch responses produced either by the serotonin 5-$HT_{2A/C}$ receptor agonist DOI (Darmani, 2001d) or SR 141716A (Janoyan et al., 2002).

5. Conclusions

The discussed results indicate that delta-9-THC prevents serotonergically mediated vomiting via mechanisms that probably involve central and peripheral as well as pre- and post-synaptic neuronal processes.

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