

The Potent Emetogenic Effects of the Endocannabinoid, 2-AG (2-Arachidonoylglycerol) Are Blocked by Δ^9 -Tetrahydrocannabinol and Other Cannabinoids

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ABSTRACT

Cannabinoids, including the endogenous cannabinoid or endocannabinoid, anandamide, modulate several gastrointestinal functions. To date, the gastrointestinal effects of the second putative endocannabinoid 2-arachidonoylglycerol (2-AG) have not been studied. In the present study using a shrew (*Cryptotis parva*) emetic model, 2-AG (0.25–10 mg/kg, i.p.) potently and dose-dependently increased vomiting frequency ($ED_{50} = 1.13$ mg/kg) and the number of animals vomiting ($ED_{50} = 0.48$ mg/kg). In contrast, neither anandamide (2.5–20 mg/kg) nor methanandamide (5–10 mg/kg) induced a dose-dependent emetogenic response, but both could partially block the induced emetic effects. Δ^9 -Tetrahydrocannabinol and its synthetic analogs reduced 2-AG-induced vomiting with the rank order potency: CP 55,940 > WIN 55,212-2 > Δ^9 -tetrahydrocannabinol. The nonpsychoactive cannabinoid, cannabidiol,

was inactive. Nonemetic doses of SR 141716A (1–5 mg/kg) also blocked 2-AG-induced vomiting. The 2-AG metabolite arachidonic acid also caused vomiting. Indomethacin, a cyclooxygenase inhibitor, blocked the emetogenic effects of both arachidonic acid and 2-AG. CP 55,940 also blocked the emetic effects of arachidonic acid. 2-AG (0.25–10 mg/kg) reduced spontaneous locomotor activity ($ED_{50} = 11$ mg/kg) and rearing frequency ($ED_{50} = 4.3$ mg/kg) in the shrew, whereas such doses of both anandamide and methanandamide had no effect on locomotor parameters. The present study indicates that: 1) 2-AG is an efficacious endogenous emetogenic cannabinoid involved in vomiting circuits, 2) the emetic action of 2-AG and the antiemetic effects of tested cannabinoids are mediated via CB_1 receptors, and 3) the emetic effects of 2-AG occur in lower doses relative to its locomotor suppressant actions.

Δ^9 -Tetrahydrocannabinol (Δ^9 -THC) is the major psychoactive constituent of the marijuana plant which is responsible for most of the pharmacological actions of cannabis. Δ^9 -THC binds with high affinity and specificity to cannabinoid receptors called CB_1 and CB_2 (Pertwee, 1999). Although the CB_1 receptor is expressed throughout the body, it is abundant primarily in the central nervous system, where it mediates the psychotropic and other effects of cannabinoids. In contrast, the CB_2 receptor is mainly found on the immune cells via which cannabinoids modulate the immune function. Both receptors belong to the superfamily of G-protein-coupled membrane receptors, inhibit adenylate cyclase and N- and Q-type calcium channel activity, and stimulate potassium channel conductance. In addition, these receptors mediate a transient elevation of intracellular free calcium concentration (Sugiura and Waku, 2000). Identification of specific cannabinoid binding sites has led to the discovery of putative

endogenous cannabinoids (endocannabinoids) (Giuffrida and Piomelli, 2000; Sugiura and Waku, 2000). At least two endocannabinoids have been recognized that produce cannabinoid-like effects: 1) arachidonylethanolamide (anandamide) was the first identified putative endocannabinoid which was originally isolated from porcine brain (Devane et al., 1992), and 2) 2-arachidonoylglycerol (2-AG) was derived from either canine gut (Mechoulam et al., 1995) or rat brain (Sugiura et al., 1995). Although the efficacy of cannabinoid agonists may vary across different loci (Breivogel and Childers, 2000), most investigators have classified cannabinoids as efficacy (2-AG; CP 55,940; WIN 55,212-2)- or affinity-driven (Δ^9 -THC; anandamide) agonists (Pertwee, 1999). However, other studies suggest that 2-AG may act as a high efficacy agonist, whereas Δ^9 -THC, WIN 55,212-2, and CP 55,940 are partial agonists (Mackie et al., 1993; Shen, 1996; Sugiura and Waku, 2000). Since the maximal effect produced by affinity-driven agonists is usually lower than that produced by efficacy-driven agonists, cannabinoids possessing partial agonist action may antagonize the maximal response produced by full agonists.

Basic and clinical studies (Mechoulam et al., 1998; Giuf-

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ABBREVIATIONS: Δ^9 -THC, Δ^9 -tetrahydrocannabinol; 2-AG, 2-arachidonoylglycerol; ANOVA, analysis of variance.

frida and Piomelli, 2000) suggest that cannabinoids are useful in treating 1) Tourette's syndrome, 2) multiple sclerosis, 3) cachexia in cancer or AIDS patients who have lost their appetite, and 4) intraocular pressure in glaucoma patients. The most well known and sustained clinical use of cannabinoids has been for the prevention of nausea and vomiting in cancer patients receiving chemotherapy (reviewed in Gralla, 1999). Until recently, the receptor mechanism by which structurally diverse cannabinoids produce their antiemetic action was not known. Studies from this laboratory have shown that low to moderate doses of the CB₁ receptor antagonist SR 141716A (1–5 mg/kg) reverses the antiemetic effects of Δ^9 -THC (Darmani, 2001b) and WIN 55,212-2 (Darmani, 2001c) against cisplatin-induced vomiting in the least shrew (*Cryptotis parva*). In addition, larger doses of SR 141716A (>10 mg/kg) were shown to induce emesis in the least shrew in a dose- and route-dependent manner (Darmani, 2001a). These findings suggest an important role for endocannabinoids in vomiting circuits.

In addition to being an excellent animal model of vomiting, the least shrew is very active and, unlike most laboratory animals, does not rest after acclimation to its environment. Thus, this species offers an opportunity to investigate the role of endocannabinoids both on locomotor activity parameters and emesis. Unlike the discussed xenobiotic cannabinoids, endocannabinoids (2-AG and anandamide) lack antiemetic activity against cisplatin (20 mg/kg)-induced emesis (unpublished observations). While we investigated the antiemetic potential of endocannabinoids against cisplatin-induced emesis, our preliminary studies indicated that 2-AG is a potent emetogenic agent. Thus, the purpose of the present study was to determine, first, whether endocannabinoids (2-AG and anandamide) can induce vomiting. Since both of these agents are rapidly metabolized, the commercially available, more stable analog of anandamide, methanandamide, was also tested. Since more stable analogs of 2-AG are not available to us, and in response to one of the reviewer's request, the possible emetic activity of the common metabolite of the cited endocannabinoids, arachidonic acid (Giuffrida and Piomelli, 2000), was also investigated. The present study was also designed to determine whether cannabinoids of diverse structure and activity (Δ^9 -THC; WIN 55,212-2; CP 55,940; and cannabidiol) can block emesis produced by endocannabinoids, whether the CB₁ antagonist/inverse agonist SR 141716A modulates emesis produced by endocannabinoids, and whether the emetic property of endocannabinoids is related to established indices of cannabimimetic activity such as reduction in motor activity parameters.

Materials and Methods

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, 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 room temperature of $21 \pm 1^\circ\text{C}$ with an ad libitum supply of food and water. The feeding and maintenance of shrews are fully described elsewhere (Darmani, 1998; Darmani et al., 1999). Δ^9 -Tetrahydrocannabinol (Δ^9 -THC), *R*-(+)-WIN 55,212-2 [*R*-(+)-[2,3-dihydro-5-methyl-3-(morpholinyl)methyl]pyrrolol [1,2,3-de]-1,4-benzoxazin-yl]-(1-naphthalenyl)methanone mesylate], arachidonylethanolamide (anandamide), arachidonic acid, indomethacin, and 2-arachidonoylglycerol were pur-

chased from Sigma/RBI (Natick, MA). SR 141716A [*N*-piperidino-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methylpyrazole-3-carboxamide] was generously given by SANOFI Recherche (Montpellier, France). CP 55,940 ((-)-*cis*-3-[2-hydroxy-4-(1,1-dimethylheptyl)phenyl]-*trans*-4-(3-hydroxy-propyl)cyclohexan-1-ol) was donated by Pfizer, Inc. (Groton, CT). All compounds were dissolved in a 1:1:18 solution of ethanol-Emulphor-0.9% saline to twice the stated drug concentrations. These concentrations were further diluted by the addition of an equal volume of saline. This procedure was necessary because the 1:1:18 vehicle mixture can cause emesis in up to 20% of animals by itself. The final vehicle mixture induced emesis only rarely. All compounds 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* (Department of Health and Human Services Publication, revised, 1985).

Emesis Studies. The present protocols were based upon our previous emesis studies in the least shrew (Darmani, 1998, 2001a,b; Darmani et al., 1999). All experiments were performed between 8:00 AM and 5:00 PM. On the test day, the 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 $20 \times 18 \times 21$ cm clean, clear plastic cage and offered four mealworms (*Tenebrio* sp.) 30 min prior to experimentation. Different groups of shrews were then injected intraperitoneally with either vehicle ($n = 8$ –11) or varying doses of 2-arachidonoylglycerol (2-AG) (0.25, 1, 2.5, 5, and 10 mg/kg; $n = 8$ –12 per group), anandamide (2.5, 5, 10, and 20 mg/kg; $n = 8$ –9 per group) methanandamide (5 and 10 mg/kg; $n = 8$ per group), or arachidonic acid (0.25, 1, 2.5, 5, and 10 mg/kg; $n = 7$ per group). Immediately following injection, each shrew was placed in the observation cage, and the frequency of vomiting (oral ejections of food or liquid; mean \pm S.E.M.) was recorded for each individual shrew for the next 30 min. Intraperitoneal administration of the cited doses of 2-AG produced emesis in the least shrew in a dose-dependent manner. Although 2-AG at 5 mg/kg caused emesis in all tested shrews, we used its 10 mg/kg dose for drug interaction studies because our preliminary studies indicated that a vehicle injection prior to 5 mg/kg 2-AG administration (i.e., control group) diluted the ability of 2-AG to produce emesis in all shrews. Thus, for drug interaction experiments, different doses of either structurally diverse cannabinoids [Δ^9 -THC (0, 1, 2.5, and 5 mg/kg; $n = 8$ per group), WIN 55,212-2 (0, 0.25, 1, and 5 mg/kg; $n = 8$ per group), CP 55,940 (0, 0.025, 0.05, and 0.1 mg/kg; $n = 8$ per group), or cannabidiol (0, 10, and 20 mg/kg; $n = 7$ –8 per group)] or the CB₁ antagonist SR 141716A (0, 1, 2.5, and 5 mg/kg; $n = 8$ –11 per group) were administered intraperitoneally to different groups of shrews 30 min prior to 2-AG (10 mg/kg, i.p.) injection. The vomiting frequency was recorded for 30 min immediately after 2-AG injection. Since Δ^9 -THC and the cited synthetic cannabinoids prevented 2-AG-induced emesis, the antiemetic potential of methanandamide (0, 2.5, 5, and 10 mg/kg) and anandamide (0, 1, 2.5, and 5 mg/kg) were also investigated in the above manner.

Since arachidonic acid potently induces emesis, some preliminary experiments were carried out following the initial review of the manuscript to reveal the possible mechanism(s) by which arachidonic acid produces emesis. Arachidonic acid can be rapidly converted by the cyclooxygenase enzyme to prostaglandins, prostacyclins and thromboxanes (Frölich, 1997). The potent inhibitor of cyclooxygenase, indomethacin [20 mg/kg, i.p.; $n = 9$, 30 min prior to arachidonic acid (2.5 mg/kg, i.p.) versus vehicle control ($n = 10$)], was tested under the experimental conditions described above to determine whether inhibition of arachidonic acid metabolism could prevent its emetic action. In addition, this dose of indomethacin was tested against 2-AG (10 mg/kg, i.p.; $n = 8$)-induced emesis. Finally, the antiemetic capacity of the synthetic cannabinoid CP 55,940 (0.025 mg/kg, i.p.; $n = 10$), was investigated against arachidonic acid (2.5 mg/kg, i.p.)-induced emesis.

Locomotor Studies. On the test day, shrews were brought in their home cages from animal quarters and were allowed to acclimate for at least 1 h to a semidark environment. The reduced light condition was necessary for the computerized video tracking, motion analysis, and behavior recognition system [Ethovision (version 2.0), Noldus Information Technology, Costerweg, Netherlands] to work efficiently. The parameters of Ethovision were set to record the following triad of locomotion activities: 1) spontaneous locomotor activity in terms of the total distance moved in meters (moving was recorded when a shrew traveled a distance greater than 2 cm in the plane of the observation cage); 2) total duration of movement in seconds (the summed time recorded for any type of movement), and 3) rearing frequency (a rearing event was recorded as a 20% decrease in surface area when shrews stand upright as seen by the overhead video camera). Our preliminary experiments indicated that a 20% change in the surface area for shrews is equivalent to 90 to 110% of manual recording of rearing frequency (Darmani, 2001b).

After acclimation to the dark laboratory environment, shrews were further acclimated in white plastic dummy observation cages (28 × 28 × 14 cm) for 30 min prior to testing. Different groups of shrews were injected intraperitoneally with either vehicle ($n = 12$) or varying doses of 2-AG (0.25, 1, 2.5, 5, and 10 mg/kg; $n = 7$ –10 per group), anandamide (2.5, 5, and 10 mg/kg, $n = 9$ –12 per group), or methanandamide (0.25, 1, 5, and 10 mg/kg; $n = 7$ –8 per group). Then each shrew was individually placed in an observation cage of the same dimension and the discussed locomotor parameters were recorded for 30 min.

Statistical Analysis. The frequency of emesis data was analyzed by the Kruskal-Wallis nonparametric one-way analysis of variance (ANOVA) and post hoc analysis by Dunn's multiple comparisons test. A p -value of <0.05 was necessary to achieve statistical significance. The incidence of emesis (number of animals vomiting) was analyzed by Fisher's exact test to determine whether there were differences between groups. When appropriate, pairwise comparisons were also made by this method. For some emesis data, the two-tailed Mann-Whitney test was used. The ID_{50} values (the inhibitory dose that prevented emesis in 50% of shrews, or the dose which reduced emesis frequency by 50%) were calculated by the use of a computerized program (GraphPad InPlot, San Diego, CA). An ANOVA, followed by Dunnett's multiple comparisons test, was used to analyze the locomotor data.

Results

Emesis Studies. Intraperitoneal administration of 2-AG caused a dose-dependent increase in the frequency of vomiting with an ED_{50} of 1.13 ± 1.24 mg/kg ($Kw_{5,47} = 23.58$, $p < 0.0003$) (Fig. 1A). Dunn's multiple comparisons post hoc test showed that relative to the vehicle-injected control group, significant enhancements (375, 413, and 350%) in the frequency of vomiting occurred in the groups injected with the 2.5 ($p < 0.05$), 5 ($p < 0.01$), and 10 ($p < 0.05$) mg/kg doses of 2-AG. In addition, Fisher's exact test showed that the percentage of shrews vomiting in response to 2-AG administration increased in a dose-dependent manner with an ED_{50} of 0.48 ± 3.5 mg/kg ($\chi^2_{5,47} = 23.42$, $p < 0.00006$) (Fig. 1B). Significant enhancements (62, 75, 100, and 83%, respectively) in the number of shrews vomiting were seen at 1 ($p < 0.03$), 2.5 ($p < 0.007$), 5 ($p < 0.002$), and 10 mg/kg ($p < 0.001$) doses of 2-AG. The second tested endocannabinoid, anandamide (2.5, 5, 10, and 20 mg/kg), failed to produce a dose-dependent emetic effect (Fig. 1). Although the Kruskal-Wallis ANOVA test indicated a significant effect for vomiting frequency ($Kw_{4,37} = 11.61$, $p < 0.02$), the post hoc test failed to show significance for any of the tested doses of anandamide. However, at 10 mg/kg it caused significant emesis in 77% ($p <$

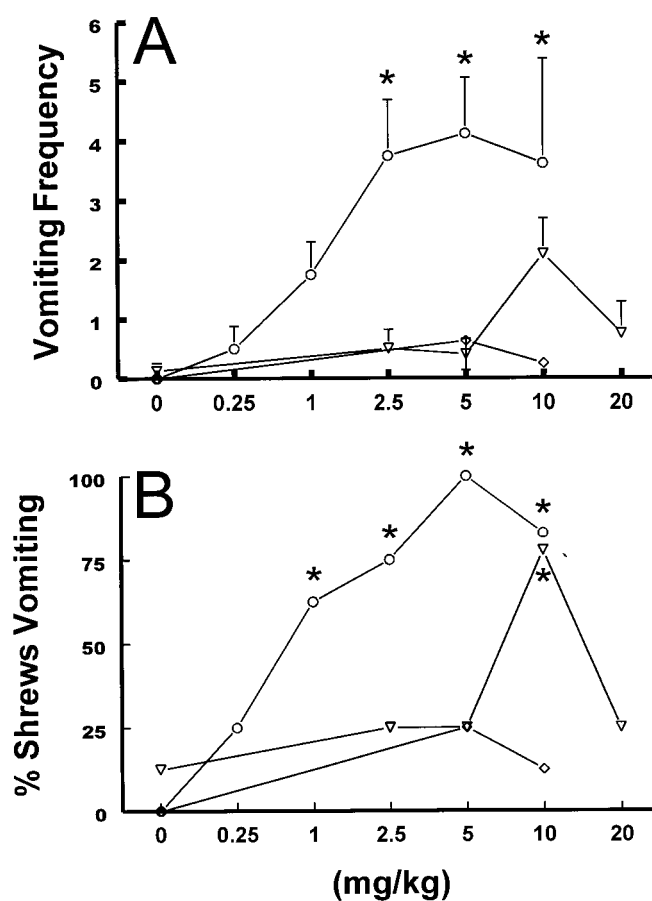


Fig. 1. Emetic dose-response effects of 2-arachidonoylglycerol (○), anandamide (▽), and methanandamide (◇) in the least shrew. A depicts the mean increase in the frequency of vomiting (\pm S.E.M.), while B shows the increase in the percentage of shrews vomiting. Emesis parameters were recorded for 30 min postinjection. *, significantly different ($p < 0.05$) from vehicle-injected control group.

0.01) of tested shrews with a mean vomiting episode of 2 ($\chi^2_{4,37} = 10.2$, $p = 0.05$). Other doses of anandamide produced emesis in less than 25% ($p > 0.05$) of shrews, whereas 12.5% of vehicle-exposed animals vomited. Furthermore, the more stable analog of anandamide, methanandamide (5 and 10 mg/kg), did not produce emesis (Fig. 1).

The metabolite of the cited endocannabinoid arachidonic acid (0, 0.25, 1, 2.5, 5, and 10 mg/kg) also caused emesis; however, the emetic effect was bell-shaped (Fig. 2). Indeed, it potently increased the frequency of vomiting ($ED_{50} = 2.3 \pm 1.99$ mg/kg) by 43 ($p > 0.05$), 329 ($p < 0.05$), 500 ($p < 0.001$), 200 ($p > 0.05$), and 71% ($p > 0.05$) ($Kw_{5,40} = 29.25$, $p < 0.001$) (Fig. 2A). Significant enhancements in the number of shrews vomiting ($ED_{50} = 0.58 \pm 2$ mg/kg) also occurred at the 1 ($p < 0.0002$), 2.5 ($p < 0.0002$), and 5 ($p < 0.007$) mg/kg doses ($\chi^2_{5,40} = 24.4$, $p < 0.00002$) (Fig. 2B).

Δ^9 -THC (1, 2.5 and 5 mg/kg) pretreatment attenuated the mean vomiting frequency [57 ($p > 0.05$), 74 ($p < 0.05$), and 97% ($p < 0.001$), respectively] induced by 2-AG (10 mg/kg) in a dose-dependent manner with an ID_{50} of 1.12 ± 1.5 mg/kg ($Kw_{3,29} = 19.47$, $p < 0.0002$) (Fig. 3A). Likewise, the percentage of shrews vomiting decreased in a dose-dependent manner [25 ($p > 0.05$), 37.5 ($p > 0.05$), and 87.5% ($p < 0.05$)] with an ID_{50} of 1.86 ± 1.52 mg/kg ($\chi^2_{3,29} = 13.97$, $p < 0.0034$) (Fig. 3B). The synthetic aminoalkylindole cannabinoid WIN

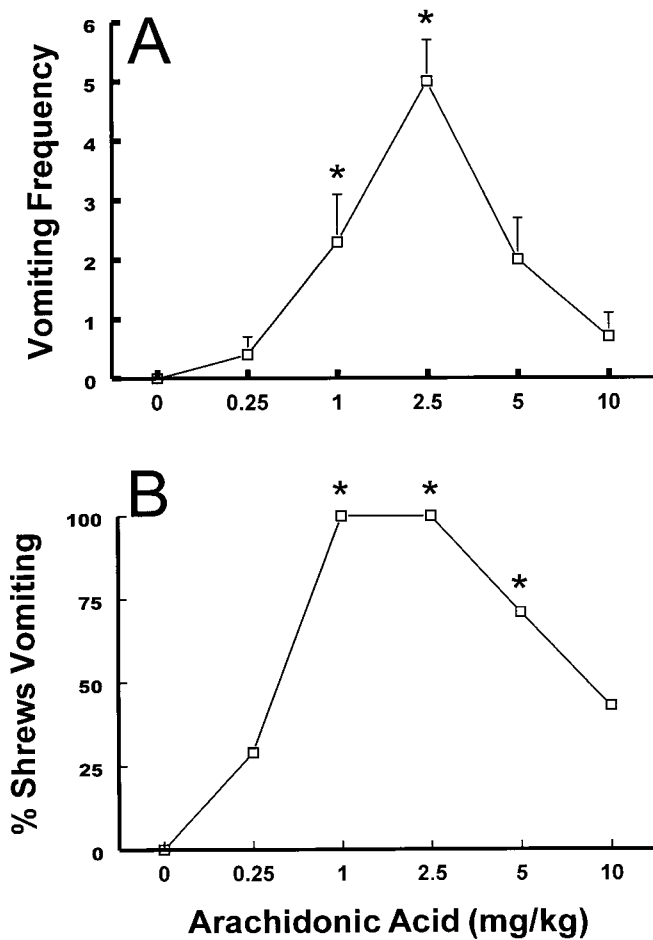


Fig. 2. Bell-shaped emetic dose-response effects of arachidonic acid in the least shrew. A shows the mean increase in the frequency of vomiting (\pm S.E.M.), while B depicts the increase in the percentage of shrews vomiting. Emesis parameters were recorded for 30 min postinjection. *, significantly different ($p < 0.05$) from vehicle-injected control group.

55,212-2 more potently reduced both the frequency of 2-AG-induced emesis ($ID_{50} = 0.16 \pm 1.36$ mg/kg) and the percentage of shrews vomiting ($ID_{50} = 0.2 \pm 2$ mg/kg) (Fig. 4). Significant attenuations in the vomiting frequency (79.9 and 97%) were observed at the 1 and 5 mg/kg doses of WIN 55,212-2 (Fig. 4A) ($Kw_{3,29} = 16.33$, $p < 0.001$), whereas a significant reduction (87.5%) in the percentage of animals vomiting was only observed at the 5 mg/kg dose (Fig. 4B). The nonclassical cannabinoid CP 55,940 was the most potent tested cannabinoid because it decreased both the vomiting frequency ($ID_{50} = 0.02 \pm 1.1$ mg/kg) and the number of shrews vomiting ($ID_{50} = 0.05 \pm 1.1$ mg/kg) at relatively low doses [($Kw_{3,29} = 19.52$, $p < 0.0002$) and ($Kw_{3,29} = 13.43$, $p < 0.004$), respectively (Fig. 5)]. The vomiting frequency was significantly reduced (77 and 94%) by 0.05 ($p < 0.05$) and 0.1 ($p < 0.001$) mg/kg doses of CP 55,940 (Fig. 5A), whereas significant blockade of animals vomiting (88.5%) occurred at the 0.1 mg/kg dose only ($p < 0.05$) (Fig. 5B). The endocannabinoid anandamide also exhibited antiemetic activity in 2-AG-treated shrews because it dose-dependently attenuated the frequency of vomiting, and a significant reduction (70%, $p < 0.05$) was observed at the 5 mg/kg dose (Fig. 6A) ($Kw_{3,29} = 10.5$, $p < 0.01$). Anandamide also significantly protected shrews from vomiting ($\chi^2_{3,29} = 10.2$, $p < 0.03$); however, the

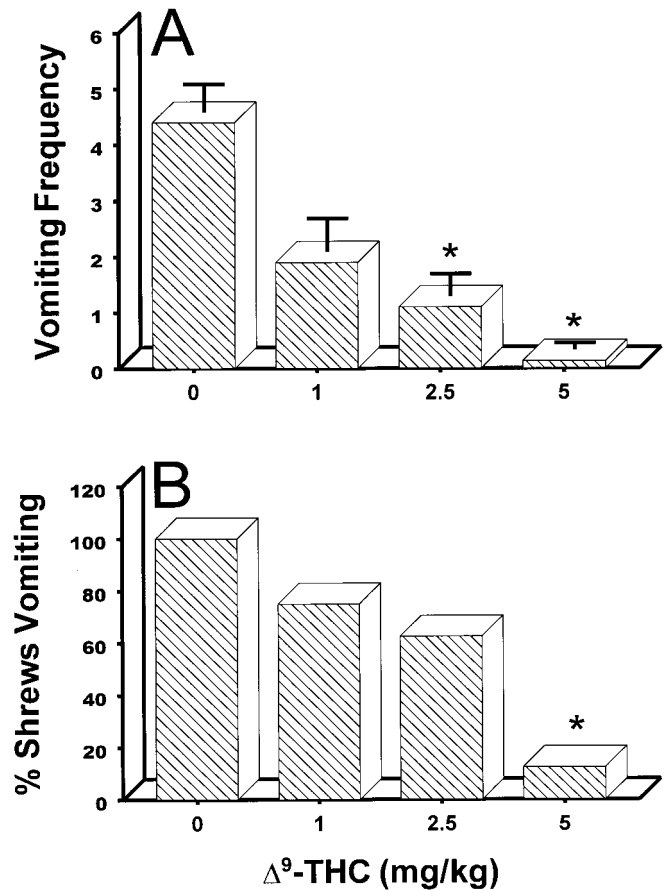


Fig. 3. Ability of Δ^9 -THC to reverse the emetic effects of a 10 mg/kg (i.p.) dose of 2-arachidonoylglycerol. The cited doses of Δ^9 -THC reduced both the frequency (mean \pm S.E.M.) of vomiting (A) and the percentage of shrews vomiting (B) in response to 2-AG administration. At zero time, shrews received Δ^9 -THC and, 30 min later, 2-AG. Emesis parameters were recorded for the next 30 min. *, significantly different ($p < 0.05$) from the vehicle-treated control group.

post hoc analysis just failed to show significance for a specific dose ($p = 0.07$) (Fig. 6B). A similar profile was seen for methanandamide (Fig. 7). Indeed, methanandamide significantly reduced the vomiting frequency (70%, $p < 0.05$) at the 10 mg/kg dose ($Kw_{3,29} = 11.06$, $p < 0.01$) (Fig. 7A). Again, although there was an overall significant protection of shrews from emesis ($\chi^2_{3,29} = 9.3$, $p < 0.03$), the post hoc test failed to indicate which dose was significant (Fig. 7B). The 10 and 20 mg/kg doses of the nonpsychoactive cannabinoid, cannabidiol, did not prevent emesis produced by 2-AG (10 mg/kg) since the cannabidiol-treated group produced, respectively, 4.3 ± 0.86 and 3.5 ± 0.6 vomits versus the vehicle-exposed control group (4.4 ± 0.5 vomits).

The CB₁ antagonist SR 141716A has been shown to produce a significant degree of emesis at 10 mg/kg or greater doses when administered intraperitoneally (Darmani, 2001a). In the present study, SR 141716A (0, 1, 2.5, and 5 mg/kg) attenuated the frequency of 2-AG-induced vomiting ($Kw_{3,33} = 14.26$, $p < 0.003$) (Fig. 8A). The cited doses of SR 141716A reduced the vomiting frequency by 59.6, 59.96, and 75.5%, and significant reductions were observed at the 2.5 and 5 mg/kg doses. SR 141716A also partially protected shrews from vomiting [37.5 ($p = 0.057$), 12.5 ($p > 0.05$) and 50% ($p < 0.02$)] [$\chi^2_{3,33} = 8.06$ ($p < 0.04$) (Fig. 8B)].

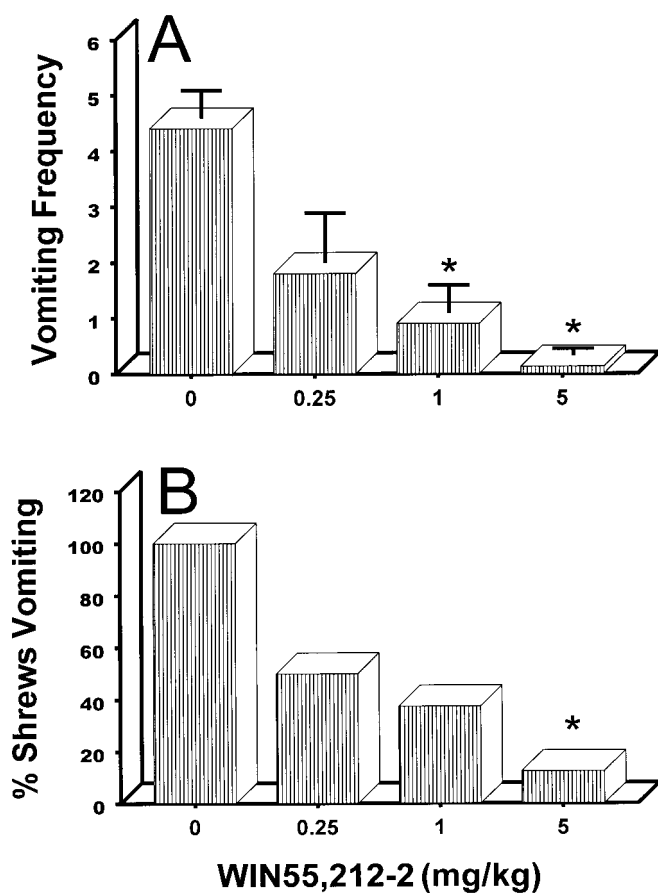


Fig. 4. Antiemetic effects of WIN 55,212-2 to reverse the emetic effects of 2-arachidonoylglycerol (10 mg/kg, i.p.). The cited doses of WIN 55,212-2 reduced both the frequency (mean \pm S.E.M.) of vomiting (A) and the percentage of shrews vomiting (B) in response to 2-AG injection. At zero time, shrews received WIN 55,212-2 and, 30 min later, 2-AG. Emesis parameters were recorded for the next 30 min. *, significantly different ($p < 0.05$) from the vehicle-treated control group.

The Mann-Whitney two-tailed test showed that the 20 mg/kg dose of indomethacin significantly reduced the mean frequency of arachidonic acid (2.5 mg/kg)-induced emesis from $3.2 (\pm 1.1)$ in the vehicle-treated control group to $0.1 (\pm 0.1)$ vomits in indomethacin-treated shrews ($U_{1,18} = 6.5$, $p < 0.006$). Furthermore, indomethacin significantly reduced the number of shrews vomiting since only one animal vomited in the indomethacin group and 9 of the 10 shrews vomited in the control group ($U_{1,18} = 9.5$, $p < 0.002$). Relative to the vehicle-treated control group ($n = 11$), indomethacin also prevented 2-AG (10 mg/kg, $n = 8$)-induced emesis since only two of the eight tested shrews vomited (2.1 ± 0.5 versus 0.3 ± 0.06 vomits) ($U_{1,18} = 19$, $p < 0.04$; and $U_{1,18} = 13$, $p < 0.01$, respectively). Finally, the synthetic cannabinoid CP 55,940 (0.025 mg/kg) blocked the ability of arachidonic acid to induce vomiting since 9 of the 10 animals vomited in the control group ($3.2 \pm$ vomits) versus 3 of 10 shrews vomiting (0.3 ± 0.15 vomits) in the CP 55,940-treated group ($U_{1,19} = 20$, $p < 0.02$; and $U_{1,19} = 16$, $p < 0.009$, respectively).

Locomotor Activity Studies. Intraperitoneal administration of the cited doses of 2-AG caused dose-dependent decreases in both spontaneous locomotor activity (i.e., total distance moved) ($ED_{50} = 10.96 \pm 2.3$ mg/kg) and rearing frequency ($ED_{50} = 4.3 \pm 2.36$ mg/kg) (Fig. 9). However, only

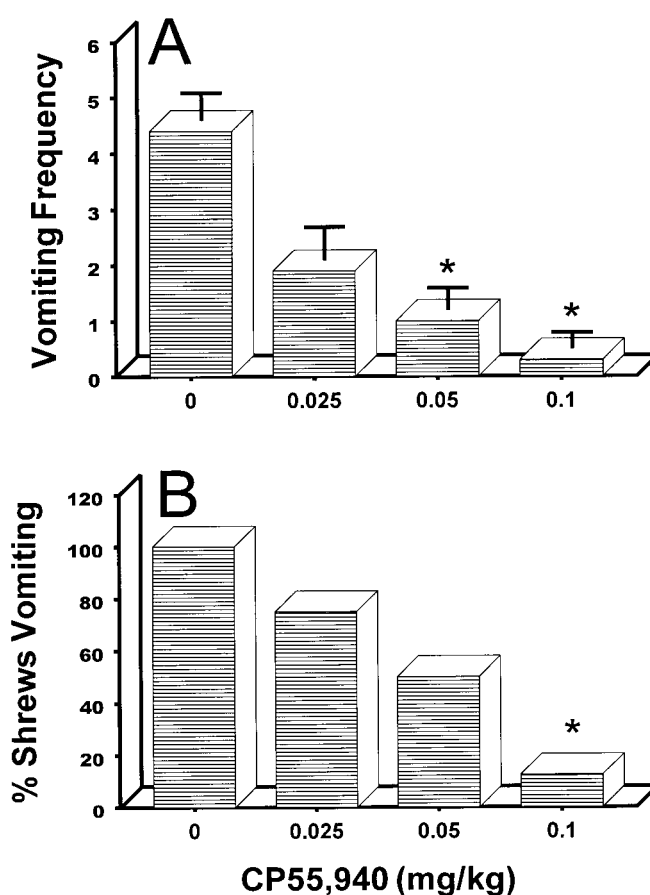


Fig. 5. Antiemetic action of CP 55,940 to reverse the emetic effects of 2-arachidonoylglycerol (10 mg/kg, i.p.). The cited doses of CP 55,940 reduced both the frequency (mean \pm S.E.M.) of vomiting (A) and the percentage of shrews vomiting (B) in response to 2-AG. At zero time, shrews received CP 55,940 and, 30 min later, 2-AG. Emesis parameters were recorded for the next 30 min. *, significantly different ($p < 0.05$) from the vehicle-treated control group.

the 10 mg/kg dose of 2-AG significantly reduced (66%, $p < 0.05$) spontaneous locomotor activity ($F_{5,41} = 3.2$, $p < 0.01$) (Fig. 9A), whereas the 5 and 10 mg/kg doses significantly attenuated [77 ($p < 0.05$) and 80.6% ($p < 0.05$)] the rearing frequency [$F_{5,41} = 5.1$, $p < 0.001$] (Fig. 9C). 2-AG administration did not affect the total duration of movement exhibited by shrews (Fig. 9B). At the doses tested, both anandamide (0, 2.5, 5, and 10 mg/kg) and methanandamide (0, 0.25, 1, 5, and 10 mg/kg) failed to significantly alter any component of the triad of motor activity exhibited by shrews (Fig. 9).

Discussion

The most important finding of the present investigation is that 2-arachidonoylglycerol (2-AG) is a potent emetogenic endocannabinoid since it increased both the vomiting frequency and the number of shrews vomiting in a dose-dependent manner, with an ED_{50} dose range of 0.48 to 1.1 mg/kg. The emetic action of 2-AG corresponds well with its presence in the intestine (Mechoulam et al., 1995). In the least shrew, both anandamide (2.5–20 mg/kg) and its more stable analog methanandamide (5–10 mg/kg) failed to produce a dose-dependent emetic effect, albeit the 10 mg/kg dose of anandamide caused significant emesis in 77% of tested shrews. This lack of dose dependence and the inability of methanandam-

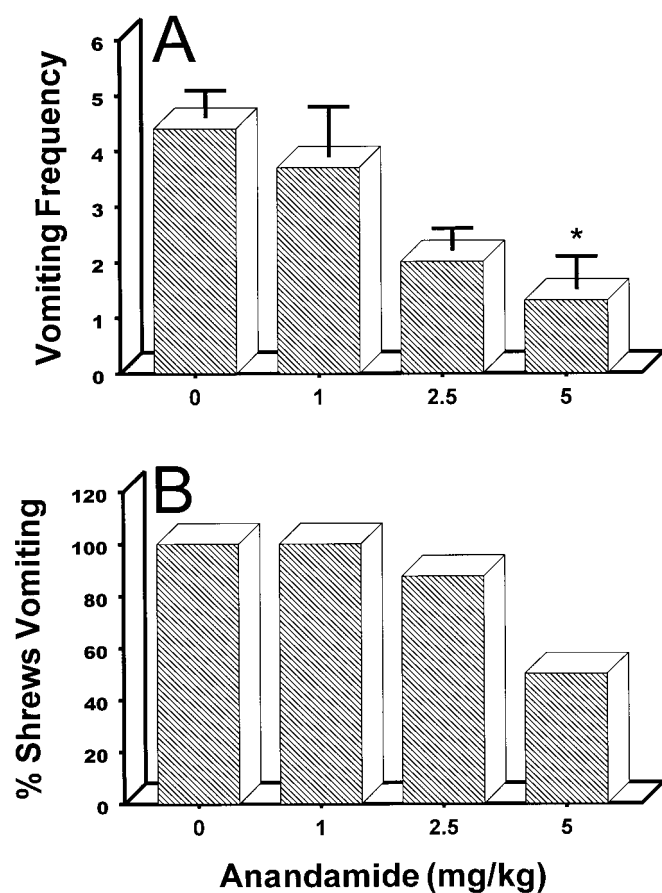


Fig. 6. Ability of anandamide to reverse the emetic effects of a 10 mg/kg (i.p.) dose of 2-arachidonoylglycerol. The cited doses of anandamide reduced the frequency (mean \pm S.E.M.) of vomiting (A). Although there was an overall significant protection of shrews from emesis, the post hoc test failed to show significance for a given anandamide dose (B). At zero time, shrews received anandamide and, 30 min later, 2-AG. Emesis parameters were recorded for the next 30 min. *, significantly different ($p < 0.05$) from the vehicle-treated control group.

ide to produce emesis supports the published in vitro and in vivo studies in mice and guinea pigs, which have shown that anandamide and methanandamide as well as Δ^9 -THC and synthetic cannabinoids dose-dependently depress peristalsis, intestinal passage of materials, and electrically stimulated intestinal contractions (reviewed in Pertwee, 2001). Furthermore, significant amounts of anandamide are also present in the rat intestine (V. Di Marzo, personal communication). However, a recent study has shown that high concentrations of anandamide do not affect electrically induced contractions of the human ileum or colon, whereas the synthetic cannabinoid WIN 55,212-2 was shown to produce potent inhibition (Manara et al., 2001).

The second major finding is that cannabinoids of diverse structure (CP 55,940, WIN 55,212-2, and Δ^9 -THC) potently attenuate 2-AG-induced increases both in emesis frequency and percentage of animals vomiting in a dose-dependent manner, with the following respective ID₅₀ potency range: 0.02 to 0.05 < 0.16 to 0.2 < 1.12 to 1.86 mg/kg. Likewise, both anandamide and methanandamide partially protected shrews from vomiting. Several hypotheses can be postulated for the antiemetic effects of both anandamide, and synthetic and plant-derived cannabinoids, and the emetic action of 2-AG. The simplest possible explanation would be that the

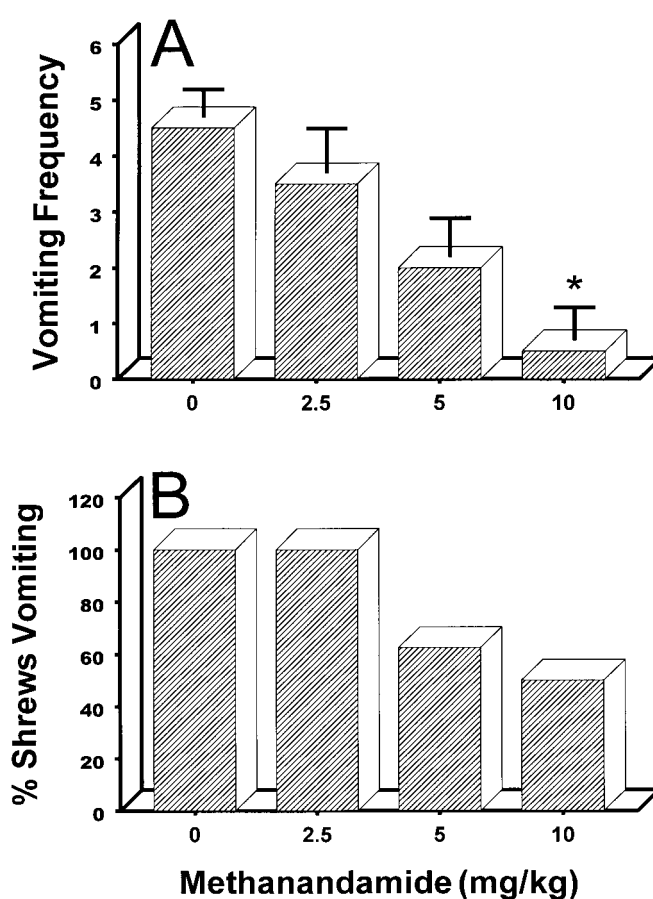


Fig. 7. Antiemetic effects of methanandamide to reverse the emetic effects of 2-arachidonoylglycerol (10 mg/kg, i.p.). The cited doses of methanandamide reduced the frequency (mean \pm S.E.M.) of vomiting (A). Although there was an overall significant protection of shrews from emesis, the post hoc test failed to show significance for a given methanandamide dose (B). At zero time, shrews received methanandamide and, 30 min later, 2-AG. Emesis parameters were recorded for the next 30 min. *, significantly different ($p < 0.05$) from the vehicle-treated control group.

cited cannabinoids act as partial agonists of cannabinoid receptors and therefore block the ability of the fully efficacious emetogenic endocannabinoid 2-AG. Support for this hypothesis comes from the ability of 2-AG to act as a highly efficacious cannabinoid CB₁ receptor agonist in producing a rapid transient increase in the intracellular free Ca²⁺ concentration, which anandamide, methanandamide, Δ^9 -THC, WIN 55,212-2, and CP55,940 nullify by acting as partial agonists (Sugiura et al., 1995; Sugiura and Waku, 2000). Several other studies also suggest that in some other test systems anandamide, CP 55,940, and Δ^9 -THC also act as partial agonists (Mackie et al., 1993; Bayewitch et al., 1996; Shen et al., 1996; Griffin et al., 1998; Shen and Thayer, 1999). In line with the notion of partial agonism, and as expected, the present study shows that nonemetic low doses of the cannabinoid CB₁ receptor antagonist SR 141716A partially antagonized the 2-AG emetic response. Larger doses of SR 141716A (Darmani, 2001a) probably produce emesis by the release of emetic neurotransmitters such as acetylcholine (Pertwee, 2001) or serotonin (Darmani and Pandya, 2000) either by inverse agonism or an as yet unknown mechanism. Indeed, cannabinoid agonists not only decrease the release and turnover of the latter emetogenic neurotransmitters

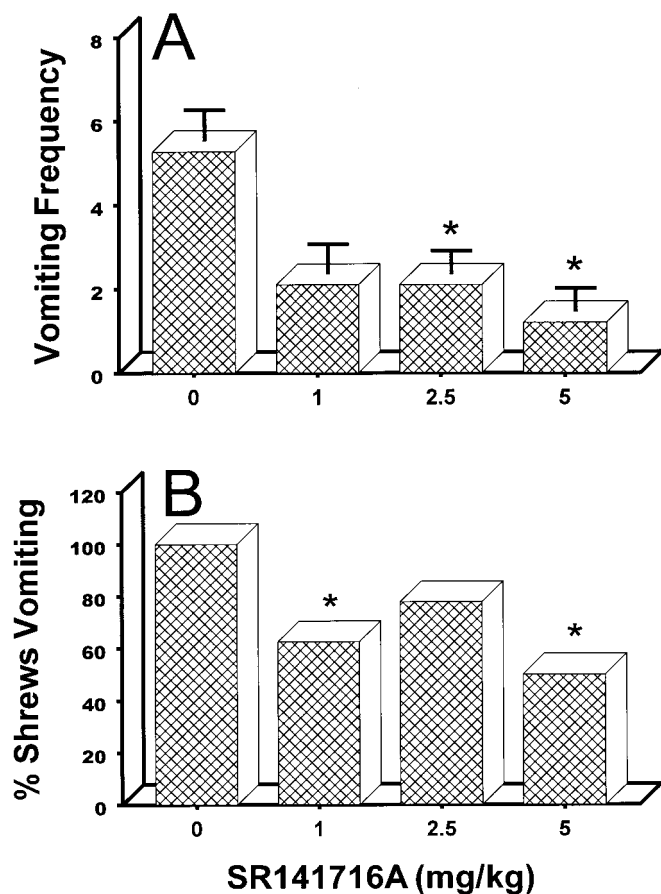


Fig. 8. Ability of the CB₁ antagonist/inverse agonist SR 141716A to reverse the emetic effects of a 10 mg/kg (i.p.) dose of 2-arachidonoylglycerol. The cited doses of SR 141716A reduced the frequency (mean \pm S.E.M.) of vomiting (graph A) but failed to completely protect shrews from vomiting (B) in response to 2-AG administration. At zero time, shrews received SR 141716A and, 30 min later, 2-AG. Emesis parameters were recorded for the next 30 min. *, significantly different ($p < 0.05$) from the vehicle-treated control group.

(Molina-Holgado et al., 1993; Pertwee, 2001) but also block intestinal contractions produced by serotonin (Pertwee, 2001). However, most cannabinoid studies classify WIN 55,212-2 and CP 55,940 as fully efficacious cannabinoids (Pertwee, 1999), which argues against the discussed partial agonist theory. A second hypothesis would be that 2-AG acts as a partial agonist of cannabinoid receptors on an endogenous emetic tone by some yet-to-be discovered endogenous cannabinoid more efficacious than both 2-AG and anandamide. This notion would explain why fully efficacious cannabinoid receptor agonists are antiemetic and block 2-AG emetogenic effects. It can also account for 1) the less potent emetogenic effects of anandamide, which, unlike 2-AG, acts also on noncannabinoid receptors that may mask its effects; 2) the lack of emetogenic effect of methanandamide, which is more efficacious than Δ^9 -THC and as efficacious as CP 55,940 (Pertwee, 1999); and 3) the emetogenic effects of large doses of SR 141716A by inverse agonism, as well as the antiemetic effects of lower nonemetogenic doses of SR 141716A (as well as anandamide and methanandamide) against 2-AG-induced vomiting. However, although 2-AG has low affinity for cannabinoid receptors, most studies show it exhibits full efficacy (Hillard, 2000). A third hypothesis for the emetic action of

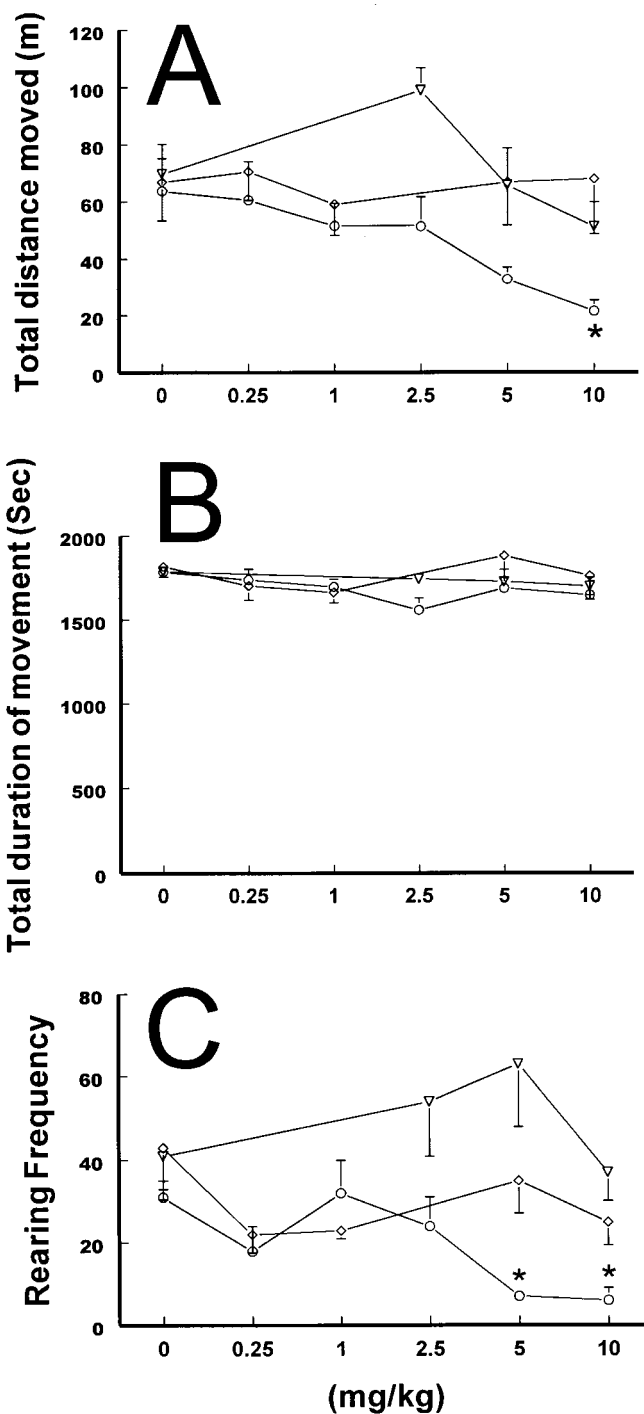


Fig. 9. Dose-response effects of the cited doses of 2-arachidonoylglycerol (○), anandamide (▽), and methanandamide (◇) on the triad of motor behaviors in the least shrew. The motor parameters [total distance moved (A), movement duration (B), and rearing frequency (C)] were recorded for 30 min by a computerized video tracking, motion analysis, and behavior recognition system (Ethovision) immediately after the administration of the cited cannabinoids. *, significantly different from vehicle-injected control group at $p < 0.05$.

2-AG would be that one or more of its metabolite(s) is/are emetogenic. 2-AG can be rapidly converted to arachidonic acid and glycerol by the enzyme fatty acid amide hydrolase (Giuffrida and Piomelli, 2000). In the present study, arachidonic acid administration caused a dose-dependent bell-shaped increase in both the incidence and frequency of vom-

iting in the least shrew. The induced vomiting is probably due to one or more metabolites of arachidonic acid as it is rapidly converted by the cyclooxygenase enzyme to a number of prostaglandins, thromboxanes, and prostacyclins (Frölich, 1997). Indeed, the cyclooxygenase inhibitor indomethacin (20 mg/kg) prevented arachidonic acid-induced vomiting in the present study. Moreover, this dose of indomethacin also blocked 2-AG-induced emesis in the least shrew. It is, however, puzzling as to why anandamide is not an efficacious emetic agent, since fatty acid amide hydrolase also converts it to arachidonic acid. It is possible that other metabolite(s) of anandamide (e.g., ethanolamine) have antiemetic properties which will block the emetic action of arachidonic acid. Furthermore, both 2-AG and anandamide are also substrates for the cyclooxygenase enzyme, and their metabolism produces different products (Kozak et al., 2000), some of which may have antiemetic activity. The inability of methanandamide to produce emesis would be explained in terms of its more stable structure, which would resist metabolism. We have further shown that a very low dose of the synthetic cannabinoid CP 55,940 (0.025 mg/kg), which was ineffective against 2-AG (10 mg/kg)-induced emesis, potently blocked arachidonic acid (2.5 mg/kg)-induced vomiting in the least shrew. The latter results indicate that antiemetic cannabinoids may 1) alter turnover of 2-AG or its products, or 2) prevent the induced emesis by stimulating antiemetic cannabinoid receptors downstream of the emetic receptors for 2-AG metabolites.

Our third finding is that 2-AG-induced emesis is probably CB₁ receptor-mediated. Indeed, the CB₁ antagonist SR 141716A (1–5 mg/kg) significantly but partially reduced both the frequency of 2-AG-induced emesis and the number of animals vomiting. This partial blockade is not an unexpected finding because larger doses of SR 141716A (≥ 10 mg/kg, i.p.; ≥ 40 mg/kg, s.c.) and not the CB₂ antagonist, SR 144528, caused emesis when administered alone (Darmani, 2001a). In the latter study, Δ^9 -THC and its analogs also blocked SR 141716A-induced emesis in an order of potency similar to that of the present investigation (CP 55,940 > WIN 55,212-2 > Δ^9 -THC). However, these cannabinoids were, respectively, 7, 20, and 14 times more effective against 2-AG- than SR 141716A-induced vomiting. SR 141716A (and not SR 144528) has also been shown to reverse the antiemetic effect of both Δ^9 -THC and WIN 55,212-2 against cisplatin-induced emesis (Darmani, 2001b,c). However, in the latter study, SR 141716A was ineffective against cisplatin-induced emesis. Cannabidiol, which has little affinity for either CB₁ or CB₂ receptors (Pertwee, 1999), neither produced emesis nor blocked the emetic action of 2-AG in the present investigation. Several in vitro and in vivo gastrointestinal functional studies in nonemetic species seem to support the current findings because SR 141716A not only was shown to reverse the depressive action of cannabinoids on intestinal motility, peristalsis, and gastrointestinal transit, but it also increased such parameters when administered alone (reviewed in Pertwee, 2001).

The emetic action of 2-AG and the antiemetic effects of the discussed cannabinoids probably involve both peripheral (e.g., myenteric plexus) and central (e.g., dorsal-vagal complex in the medulla) CB₁ receptors (reviewed in Pertwee, 2001). Indeed, these studies have shown that either intracerebroventricular or direct administration of Δ^9 -THC to the

dorsal surface of the medulla reduced gastrointestinal function. Furthermore, ganglion blockade or vagotomy was shown to block the gastrointestinal effects of systemically administered Δ^9 -THC. Within the myenteric plexus of the gut, cannabinoid CB₁ receptor agonists inhibited electrically evoked contractions of guinea pig small intestine by presynaptic inhibition of acetylcholine release (Pertwee, 2001). These peripheral and central structures contain significant amounts of CB₁ receptors or its mRNA (Herkenham et al., 1991; Kulkarni-Narla and Brown, 2000; Mailleux and Vanderhaeghen, 1992). Moreover, 2-AG is found both in the gastrointestinal tract and in the brain, and the highest level of 2-AG is present in the brain stem (Mechoulam et al., 1995; Bisogno et al., 1999). The motor-inhibitory effects of cannabinoids are also shown to be CB₁ receptor-mediated, but CB₁ receptors responsible for cannabinoid locomotor inhibition are located in the basal ganglia and its subcortical structures (Sañudo-Peña et al., 1999, 2000; Darmani, 2001a,b). In the present investigation anandamide or methanandamide (0.25–10 mg/kg) did not alter the triad of locomotor parameters, whereas 2-AG (0.25–10 mg/kg) reduced spontaneous locomotor activity ($ED_{50} = 10.96$ mg/kg) and the rearing frequency ($ED_{50} = 4.3$ mg/kg) in a dose-dependent manner. However, other studies in rodents have shown that larger doses of 2-AG and anandamide are motor suppressive, and both agents are equipotent ($ED_{50} = 13$ – 18 mg/kg) in reducing spontaneous locomotor activity (Mechoulam et al., 1995). The present study further shows that reduction in locomotor parameters requires larger doses of 2-AG and is in the opposite direction to 2-AG enhancement of vomiting.

In summary, 2-AG and not anandamide (or methanandamide) is the endocannabinoid in emetic circuits that potently produces emesis. Although some of the discussed data are preliminary and further experiments are ongoing in this laboratory, the present results suggest that the tested antiemetic cannabinoid agonists prevent 2-AG-induced vomiting by: 1) possibly affecting the metabolic conversion of 2-AG, arachidonic acid, or its metabolites; 2) acting as antiemetic agonists on cannabinoid CB₁ receptors in some emetic circuits downstream of the receptor sites for 2-AG metabolites; 3) directly blocking the emetic action of 2-AG on cannabinoid receptors by behaving as partial agonists against the full agonist nature of 2-AG; or 4) acting as full agonists by nullifying the partial agonist action of 2-AG on an antiemetic endogenous tone. The emetic action of 2-AG appears to be CB₁ receptor-mediated inasmuch as SR 141716A reduced the frequency of emesis but failed to totally protect the shrews from vomiting. At moderate doses, 2-AG (but not anandamide nor methanandamide) also reduces locomotor parameters. However, relative to production of emesis, locomotor suppression requires larger doses of 2-AG. Thus, these findings indicate that the emetic and motor suppression of 2-AG are CB₁ receptor-mediated but occur at different loci.

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