

## The Cannabinoid Agonist WIN55,212-2 Suppresses Opioid-induced Emesis in Ferrets

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**Background:** Cannabinoid receptor agonists reverse nausea and vomiting produced by chemotherapy and radiation therapy in animals and humans but have not been tested against opioid-induced emesis. This study tests the hypothesis that cannabinoid receptor agonists will prevent opioid-induced vomiting.

**Methods:** Twelve male ferrets were used. They weighed 1.2–1.6 kg at the beginning and 1.8–2.3 kg at the end of the experiments. All drugs were injected subcutaneously. WIN55,212-2, a mixed CB1–CB2 cannabinoid receptor agonist, was administered 25 min before morphine. Retches and vomits were counted at 5-min intervals for 30 min after morphine injection.

**Results:** Retching and vomiting responses increased with increasing morphine doses up to 1.0 mg/kg, above which the responses decreased. Previous administration of naloxone prevented morphine-induced retching and vomiting. WIN55,212-2 dose-dependently reduced retching and vomiting. The ED<sub>50</sub> was 0.05 mg/kg for retches and 0.03 mg/kg for vomits. At 0.13 mg/kg, retching decreased by 76% and vomiting by 92%. AM251, a CB1 receptor-selective antagonist, blocked the antiemetic actions of WIN55,212-2, but AM630, a CB2 receptor-selective antagonist, did not.

**Conclusions:** These results demonstrate that WIN55,212-2 prevents opioid-induced vomiting and suggest that the antiemetic activity of WIN55,212-2 occurs at CB1 receptors. This is consistent with findings that CB1 receptors are the predominant cannabinoid receptors in the central nervous system and that antiemetic effects of cannabinoids appear to be centrally mediated.

NAUSEA and vomiting are common and troubling side effects of opioid analgesics.<sup>1,2</sup> Although drugs exist for the control of nausea and vomiting from a variety of causes, no therapy has been conclusively demonstrated to be effective in preventing opioid-induced emesis. For example, studies of ondansetron activity against opioid-induced nausea and vomiting have yielded mixed results, both in the ferret and in humans.<sup>3–6</sup> Cannabinoid receptor agonists have been demonstrated to be effective antiemetics in some clinical settings<sup>7</sup> but have not been tested against opioid-induced emesis. Using cannabinoid receptor agonists in combination with opioid receptor agonists represents a potentially attractive therapeutic approach because cannabinoids have analgesic proper-

ties and have been suggested to synergize with opioids in producing analgesia.<sup>8,9</sup>

Cannabinoid receptor agonists have been demonstrated to reverse the nausea and vomiting produced by chemotherapeutic agents in animals and in humans.<sup>7,10–12</sup> They have also been shown to reverse nausea and vomiting in patients receiving radiation therapy.<sup>13</sup> Chemotherapy-induced emesis has mechanistic similarities with opioid-induced emesis, suggesting that cannabinoids may be effective against emesis caused by opioids. In particular, chemotherapeutic agents have central emetic actions in the brainstem, where the area postrema–nucleus tractus solitarius plays an essential role.<sup>14</sup> Like opioids, chemotherapeutic agents also have peripheral actions in the gastrointestinal tract. Chemotherapeutic agents are hypothesized to release 5-hydroxytryptamine from enterochromaffin cells, which then activates the vagus nerve *via* 5-hydroxytryptamine 3 receptors.<sup>15</sup> However, there are also differences between chemotherapy- and opioid-induced vomiting, suggesting that an agent that is effective against one will not necessarily be active against the other. The 5-hydroxytryptamine 3 receptor antagonist, ondansetron, is effective in ameliorating chemotherapy-induced vomiting in patients and animal models.<sup>16,17</sup> However, studies of ondansetron activity against morphine-induced vomiting in the ferret and in patients have yielded mixed results. In addition, a role of ascending input from the vagus nerve in chemotherapy-induced vomiting is suggested by observations that vagotomy reduced cisplatin-induced emesis in the ferret.<sup>15,18</sup> However, vagotomy had no effect on opioid-induced emesis.<sup>3</sup> The mechanistic similarities between opioid-induced vomiting and chemotherapy-induced vomiting suggest that cannabinoids may be effective against opioid-induced vomiting. However, the differences in mechanism made this prediction uncertain. The current study directly tests the hypothesis that cannabinoid receptor agonists can prevent opioid agonist-induced vomiting by testing the ability of WIN55,212-2, a mixed CB1–CB2 cannabinoid receptor agonist, to prevent morphine-induced retching and vomiting in the ferret.

### Methods

#### Animals

This protocol was approved by the University of Arizona Animal Care and Use Committee. Male ferrets (N = 12), purchased from Marshall Farms (North Rose, NY) and weighing 1.2–1.6 kg at the beginning of the study and 1.8–2.3 kg at the completion of the study, were

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housed two per cage with a 12-h light-dark cycle. The same animals ( $N = 12$ ) were used for each experiment. Ferrets were given food and water *ad libitum*. They were acclimated for at least 2 weeks in the animal care facility before the experiments.

#### *Drug Preparation*

Morphine sulfate was obtained from Sigma Laboratories and dissolved in normal saline. WIN55,212-2 was obtained from Sigma Laboratories and dissolved in dimethyl sulfoxide. AM251, a 300-fold selective CB1 receptor antagonist, and AM630, a 70- to 165-fold selective CB2 receptor antagonist, were dissolved in dimethyl sulfoxide. The doses of AM251 and AM630 used (2.5 mg/kg) were selected because they were effective at antagonizing the analgesic effects of cannabinoid receptor agonists in rats (M. M. Ibrahim and T.P. Malan, Jr., Ph.D., M.D., unpublished observations, March 2000). Naloxone was obtained from Research Biochemicals International and dissolved in normal saline.

#### *Drug Administration and Testing*

Ferrets were acclimated for 30 min in individual Plexiglas cages. They were anesthetized with 4% halothane in air using a cone mask until loss of righting occurred. Once anesthetized, ferrets were weighed, and the dorsal nape of the neck was shaved. A patch of fur was marked using bromophenol blue for identification of each ferret. Injections were performed subcutaneously on the dorsal aspect of the neck. Drugs were injected in volumes from 0.3 to 0.6 ml. WIN55,212-2 was administered 25 min before morphine, and naloxone was administered 10 min before morphine. Saline was used as a vehicle control for morphine, and dimethyl sulfoxide was used as a vehicle control for WIN55,212-2. Duration of anesthesia was 1 min or less, and complete recovery occurred in less than 1 min. Animals were observed for the 30 min after morphine injection, and retches and vomits were recorded. Retching was defined as any rhythmic abdominal contraction without expulsion, whereas vomiting was defined as any oral expulsion episode. Total retches and vomits were then recorded for each animal. An interval of at least 3 days was allowed between testing of each animal to allow for drug washout and to minimize the development of tolerance.

#### *Statistical Analysis*

Data were analyzed dichotomously. For examination of the incidence of retching or vomiting, a binary variable was created for whether an animal retched or vomited. The binary data was analyzed by logistic regression. For examination of the number of retches or vomits per animal, the count data were analyzed by negative binomial regression. The negative binomial regression method was required because of overdispersion of the data. All analyses controlled for the lack of independent data, because of repeated use of the same animals, by calculating robust

variance estimates. Linear combination after logistic or negative binomial regression was used for comparisons between different doses of morphine or WIN55,212-2. Statistical analysis was performed using Intercooled Stata 6.0 (Stata Corporation, College Station, TX).  $A_{50}$  values (the dose producing a 50% effect) for WIN55,212-2 were determined from dose-response curves using GraphPad Prism 3.0 (GraphPad Software, San Diego, CA).

## Results

### *Morphine-induced Retching and Emesis*

One ferret retched once after halothane alone. Although each animal retched or vomited at one or more morphine doses, not all animals retched or vomited at each individual dose. The percentage of ferrets that retched was significantly increased for all morphine doses ( $P < 0.05$ ) and reached a maximum (92%) at a dose of 0.6 mg/kg (fig. 1). The percentage of animals that vomited tended to increase at all morphine doses and reached a maximum of 67% at 0.6 mg/kg, but this increase was not significant ( $P = 0.06$  at 0.6 mg/kg morphine). The mean number of retches and vomits per animal was significantly increased for all doses of morphine given, with the mean number of retches or vomits peaking at 1 mg/kg ( $17 \pm 5$  and  $2.1 \pm 1.1$ , respectively; fig. 1). As a result, 1 mg/kg morphine was used in all antiemetic experiments. At greater than 1 mg/kg morphine, the number of retches and vomits per animal decreased, with 1.5 mg/kg producing 62% fewer retches and 80% fewer vomits than 1.0 mg/kg ( $P = 0.07$  and  $0.001$ , respectively). With 1 mg/kg morphine, the greatest number of retches and vomits occurred in the first 5-min interval, and retching and vomiting were complete by 15 min. Pretreatment with naloxone (0.1 mg/kg administered subcutaneously) reduced the number of morphine-induced retches per animal by 96% and vomits per animal by 92% (fig. 2). The percentage of animals retching decreased from 67 to 17% with naloxone pretreatment, and the percentage of animals vomiting decreased from 41 to 17%. These decreases were not statistically significant.

### *Cannabinoid Suppression of Morphine-induced Retching and Vomiting*

Pretreatment with WIN55,212-2 reduced the average number of retches per animal by up to 76% and the average number of vomits per animal by up to 92% ( $P$  values for linear trends were 0.002 for retches and 0.001 for vomits; fig. 3). The  $A_{50}$  for retches was 0.03 mg/kg and for vomits was 0.05 mg/kg. However, WIN55,212-2 did not significantly decrease the number of ferrets that retched or vomited compared with animals receiving morphine alone (fig. 3). Dimethyl sulfoxide alone had no effect on morphine-induced retching and vomiting.

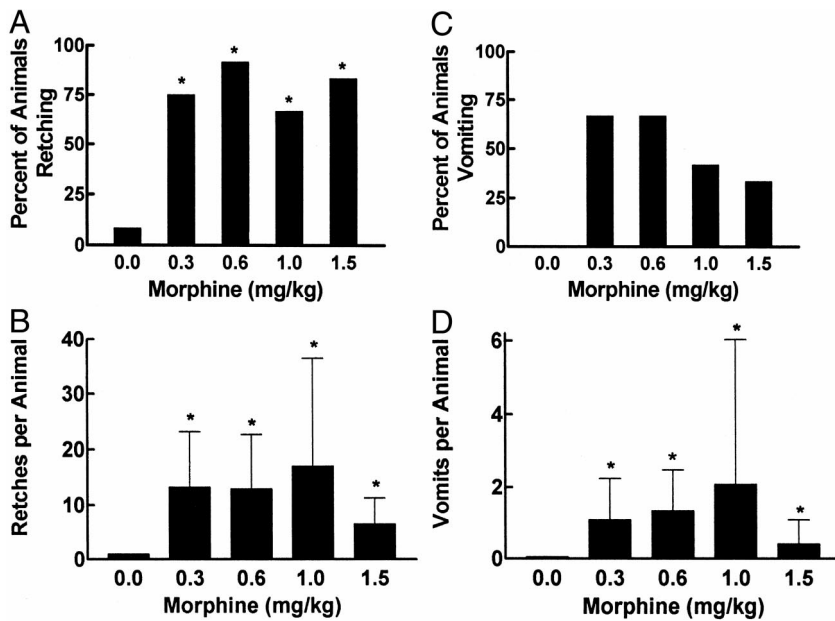


Fig. 1. Percentage of animals retching (A) and vomiting (B) and average number of retches (C) and vomits (D) per animal after morphine administration. Number of retches or vomits per animal are displayed as mean  $\pm$  SD (N = 12). \* $P < 0.05$  compared with animals not receiving morphine.

AM251 (2.5 mg/kg) blocked WIN55,212-2 (0.13 mg/kg) suppression of morphine (1 mg/kg)-induced retches and vomits (fig. 4). Treatment with AM251 resulted in a fourfold increase in retches and an eightfold increase in vomits compared with the combination of WIN55,212-2 and morphine ( $P = 0.001$ ). AM630 (2.5 mg/kg) did not block WIN55,212-2 suppression of retching and vomiting (fig. 4;  $P = 0.4$  and  $0.6$ , respectively).

## Discussion

Morphine caused retching and vomiting in ferrets. This response was prevented by pretreatment with naloxone, demonstrating that the retching and vomiting is mediated by opioid receptors. Retching and vomiting responses increased with increasing morphine doses up to 1.0 mg/kg, above which the responses decreased. This dose-response curve shape was previously observed with loperamide-induced retching and vomiting in ferrets and with morphine-induced vomiting in dogs and in ferrets.<sup>3,4,19</sup> The loss of the emetic response at higher morphine doses was hypothesized to be caused by antiemetic effects of opioid receptors activated at higher doses. Antiemetic effects of opioids have been demon-

strated previously.<sup>20</sup> Our observation of maximum retching and vomiting at 1.0 mg/kg morphine appears to contrast with the data of Wynn *et al.*,<sup>4</sup> who observed a maximum at 0.3 mg/kg. However, the general shapes of the dose-response curves were similar, with an initial increase, a broad peak, and a subsequent decline. The difference in the doses of morphine producing a peak effect can be explained by the breadth of the peak and experimental variation at each dosage point.

Opioid-induced nausea and vomiting is a significant clinical problem. In a pain clinic population, 8–18% of patients suffered nausea and 23–40% of patients experienced vomiting after receiving opioids.<sup>1</sup> In hospitalized nonsurgical patients, opioids caused nausea in 35% of patients, vomiting in 14%, and retching in 7%.<sup>2</sup> Nausea and vomiting can impair functional status and lead to significant alterations in patients' perceptions of quality of life.<sup>2,21</sup>

Opioids have central actions in the chemoreceptor trigger zone (area postrema), where they trigger brainstem emetic mechanisms. The area postrema is essential for opioid-induced vomiting, as demonstrated by the observations that, in dogs and ferrets, ablation of the area postrema eliminates the emetic response to opi-

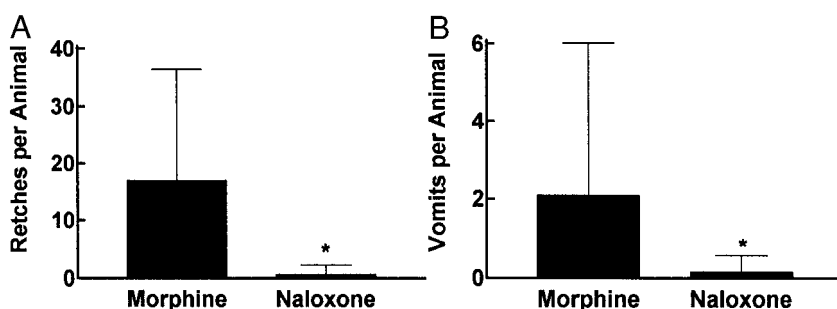
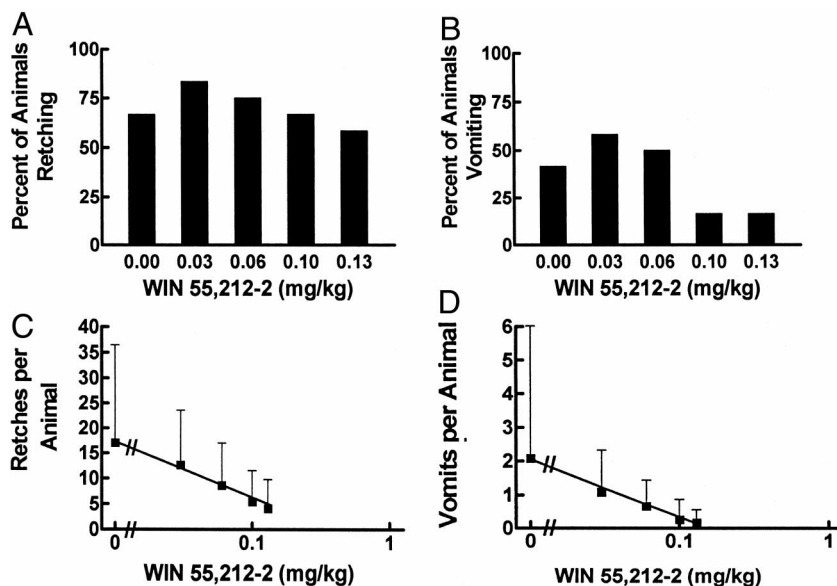


Fig. 2. Effect of naloxone (0.1 mg/kg) on retching (A) and vomiting (B) induced by 1 mg/kg morphine. Data displayed as mean  $\pm$  SD (N = 12). \* $P < 0.05$  compared with morphine alone.

Fig. 3. WIN55,212-2 suppression of retching and vomiting induced by 1 mg/kg morphine. Percentage of animals retching (A) and vomiting (B) and average number of retches (C) and vomits (D) per animal (N = 12). The effect of WIN55,212-2 on the number of retches per animal on the number of vomits per animal was significant ( $P = 0.002$  and  $0.001$ , respectively) as assessed by the dose-response relation. Differences in the percentages of animals retching or vomiting were not significant.

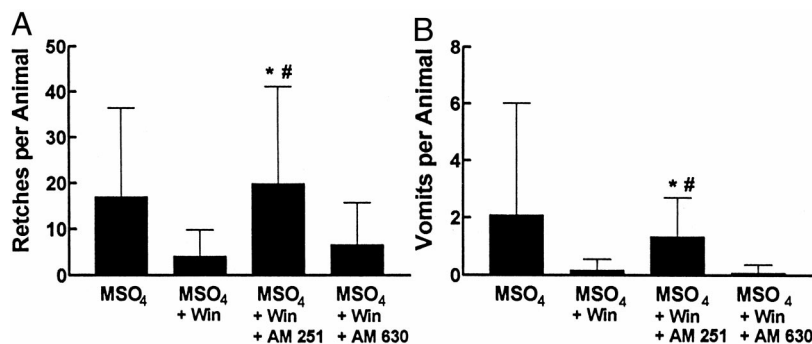


oids.<sup>3,19</sup> In dogs, the emetic effect of morphine was blocked by methylnaltrexone, a quaternary opioid antagonist with peripherally restricted action, suggesting that morphine-induced emesis is produced by structures outside the blood-brain barrier.<sup>22</sup> The blood-brain barrier is not complete in the area of the area postrema; therefore, this locus can be considered pharmacologically peripheral. Opioids may also have antiemetic actions at brainstem sites.<sup>19,20,23</sup> In dogs, pretreatment with methylnaltrexone and morphine reduced apomorphine-induced emesis and blocked cisplatin-induced emesis,<sup>24</sup> suggesting that methylnaltrexone blocked the peripheral emetic effect of morphine, thereby unmasking its central antiemetic effect. In addition to central emetic and antiemetic mechanisms, opioids have peripheral actions in the gastrointestinal tract that are primarily manifested as decreased propulsion and motility.<sup>25</sup> These could potentially contribute to nausea and vomiting by activating gastrointestinal mechanoreceptors. These mechanoreceptors respond to overdistension or disordered motility by sending ascending signals to the brainstem vomiting center *via* the vagus nerve.<sup>26</sup> However, loperamide-induced emesis in the ferret was not altered by abdominal vagotomy, suggesting that vagal input may not be important.<sup>3</sup>

WIN55,212-2 abolished morphine-induced retching and vomiting in a dose-dependent fashion. The antiemetic activity of WIN55,212-2 was blocked by AM251, a CB1-selective cannabinoid receptor antagonist,<sup>27</sup> but not by AM630, a CB2-selective cannabinoid receptor antagonist-inverse agonist,<sup>28</sup> suggesting that the antiemetic actions of WIN55,212-2 occur *via* the CB1 receptor. This is consistent with the finding that CB1 receptors are the predominant cannabinoid receptors in the central nervous system and the fact that the antiemetic effects of cannabinoids are believed to be centrally mediated.<sup>29,30</sup> The details of the antiemetic mechanism of cannabinoids have not been fully elucidated. Because cannabinoids are active in blocking emesis produced by centrally acting drugs, it has been proposed that they act by inhibiting the action of the vomiting center in the medulla.<sup>10</sup>

AM251 was used as a CB1 receptor-selective antagonist because it is 300-fold selective for the CB1 receptor compared with the CB2 receptor in receptor-binding experiments. In addition, in *in vivo* tests of analgesia in rats, AM251 did not antagonize the effects of a CB2 receptor-selective agonist (M. M. Ibrahim, A. Makriyanis, and T. P. Malan, Jr., unpublished observations, March 2000). AM630 was used as a CB2 receptor-selective

Fig. 4. Suppression by WIN55,212-2 (0.13 mg/kg) of retching (A) and vomiting (B) induced by morphine (MSO<sub>4</sub>, 1 mg/kg) is reversed by AM251 (2.5 mg/kg) but not by AM630 (2.5 mg/kg) (N = 12). \* $P < 0.05$  compared with morphine plus WIN55,212-2. # $P < 0.05$  compared with morphine plus WIN55,212-2 plus AM630.



tive antagonist because it is 70- to 165-fold selective for the CB2 receptor compared with the CB1 receptor in receptor-binding experiments. It has also been suggested to have weak activity at the CB1 receptor in stably transfected cells and inverse agonist activity at the CB2 receptor in transfected cells.<sup>31</sup> However, interpretation of actions in transfected cells is complicated by the fact that receptor number may be significantly higher in these cells than in native tissue. As AM630 had no effect on WIN55,212-2 suppression of morphine-induced vomiting, while AM251 did, AM630 appears to be selective for the CB2 receptor *in vivo*.

The fact that individual animals were reused multiple times for drug testing raises the possibility that some of the effects observed were caused by incomplete washout of drug or by tolerance to opioid or cannabinoid effects. However, we do not believe this is likely for two reasons. First, animals were allowed a 3-day recovery period between testing to allow drug washout and to prevent the development of tolerance. Second, although drugs were typically administered at 3-day intervals, selected doses were repeated at longer (7-14-day) intervals, and the results were similar to those obtained with the more frequent dosing scheme.

We cannot completely rule out the possibility that the decreases in vomiting and retching observed with WIN55,212-2 were caused by motor effects of the drug. However, all animals were able to walk and stand after receiving WIN55,212-2. The one significant behavioral observation after administration of the combination of morphine and WIN55,212 was that the animals were sedated.

The ferret has gained acceptance as an animal model for the study of nausea and vomiting. For example, ferrets given cisplatin display two distinct phases of emesis similar to those observed in humans.<sup>32</sup> The late phase of emesis observed with cisplatin was not tested in this study because only one phase of emesis has been observed with opioids. The ferret responds to a variety of emetogens, including cytotoxic drugs, radiation, and opioids.<sup>3,4,33</sup> Antiemetic drugs, such as ondansetron, reduce the emetic response to cytotoxic drugs in ferrets,<sup>17</sup> as they do in humans.<sup>16</sup> In contrast, studies of ondansetron activity against opioid-induced nausea and vomiting have yielded mixed results, both in ferrets and in humans.<sup>4-6</sup> There are two principal differences known between humans and ferrets that limit extrapolation of data from ferrets to humans: the lack of anticipatory emesis in ferrets, important when studying chemotherapy-induced emesis, and pharmacokinetic differences between the two species, which make the comparison of drug doses difficult.<sup>26</sup> The synergistic interaction between opioids and cannabinoids suggests that analgesia may be produced with a combination of low doses of each, possibly leading to reduced side effects compared with an equianalgesic dose of each drug alone. However, this

benefit may be minimized if the drugs synergize in producing side effects. Like opioids, cannabinoids decrease gastrointestinal motility and cause respiratory depression.<sup>34-36</sup> It is not known whether these actions are subadditive, additive, or synergistic. It is also not clear what the addictive potential of an opioid-cannabinoid combination would be, although the risk of addiction with the medical use of opioids is low, and cannabinoids may have less addictive potential than opioids.<sup>36</sup>

A mixed CB1-CB2 cannabinoid receptor agonist reversed the retching and vomiting produced by morphine in the ferret. This suggests that cannabinoids may be effective in treating opioid-induced nausea and vomiting in patients. This finding, in combination with evidence that cannabinoids and opioids may act synergistically in producing analgesia,<sup>9</sup> suggests that using these drugs in combination may have clinical utility.

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