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Antiemetic Effects of 5-HT_{1A} Agonists in the Pigeon

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WOLFF, M. C. AND J. D. LEANDER. *Antiemetic effects of 5-HT_{1A} agonists in the pigeon.* PHARMACOL BIOCHEM BEHAV 49(2) 385–391, 1994. – Ditolyguanidine (DTG) induced a dose-dependent emetic response in pigeons, with 100% of the birds vomiting after 5.6 mg/kg. Retching and vomiting originally induced by DTG could be conditioned to the test situation. Both the unconditioned and conditioned emetic responses were dose-dependently blocked by 8-hydroxy-(di-n-propylamino)tetralin (8-OH-DPAT) and LY228729, agonists at the 5-HT_{1A} subtype of serotonin receptor, but not by the 5-HT_3 antagonist tropisetron. Higher doses (0.25–0.5 mg/kg) of tropisetron exhibited intrinsic emetic activity which could also be prevented by 8-OH-DPAT. NAN-190, a putative 5-HT_{1A} partial agonist, produced both an antiemetic response when administered before DTG and also attenuated the antiemetic effects of 8-OH-DPAT. Pentobarbital blocked the conditioned, but not the unconditioned DTG-induced emesis. These results support the possibility that 5-HT_{1A} agonists exhibit antiemetic activity against a broad range of emetic stimuli, including conditioned vomiting which is usually resistant to pharmacological attenuation.

Emesis Anticipatory vomiting Pigeon 5-HT_3 antagonist 5-HT_{1A} agonist DTG NAN-190

VARIOUS compounds that have activity at serotonin (5-HT) receptor subtypes are effective antiemetics in laboratory animals. For instance, 5-HT_3 antagonists block vomiting produced by radiation and cytotoxic drugs in ferrets (28). However, the 5-HT_3 antagonists may have a limited range of usefulness in that they are ineffective in the treatment of emesis caused by other types of stimuli, such as apomorphine (28), xylazine, and motion (26), which are thought to produce emesis through central mechanisms (3). In contrast, the selective 5-HT_{1A} agonist 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT) blocks emesis induced by motion, xylazine, and the chemotherapy agent cisplatin in cats (25). Likewise, buspirone, a partial 5-HT_{1A} agonist, blocks apomorphine-induced emesis in dogs (2), as well as cisplatin-induced emesis in cats (25).

Emesis induced by apomorphine, copper sulfate, piperazine, cyclizine (23), and cisplatin (34) has been previously studied in pigeons. Hudzik (19) recently reported that ditolyguanidine (DTG) reliably evoked vomiting in the pigeon. DTG is considered to be a selective ligand for sigma receptors that are widely distributed in the brain with concentrations in limbic structures and brain stem areas that serve motor functions (40). DTG-induced emesis may be mediated by these receptors, as other sigma ligands such as dextromethorphan and (+)-3-(3-hydroxyphenyl)-N-(1-propyl)-piperidine ((+)-3-PPP) also induce vomiting in the pigeon (19). Furthermore, the DTG-induced emetic response is antagonized by haloperidol and BMY14802 (19), which also have some selectivity for sigma sites and are thought to function as antagonists (40).

In view of the apparent broad spectrum antiemetic effects observed with 5-HT_{1A} agonists in cats, the present investigation was undertaken to determine if the 5-HT_{1A} agonists 8-OH-DPAT and LY228729 [(−)-4-(dipropylamine)-1,3,4,5-tetrahydrobenz-(c,d)indole-6-carboxamide] (7,14) and the 5-HT_{1A} partial agonist, NAN-190 (12,15,16,18,36) would also prevent vomiting induced by DTG in pigeons. NAN-190 was also tested as an antagonist of the antiemetic actions of 8-OH-DPAT. In addition, 8-OH-DPAT was injected chronically to determine if tolerance would develop to its antiemetic effects.

During the course of this study, it soon became obvious that some of the pigeons were beginning to vomit during the test situation even before the DTG injection; that is, these birds were exhibiting conditioned vomiting. The Russian physiologist Pavlov (32) was the first to demonstrate that laboratory animals, in his case dogs, could be conditioned to vomit by pairing a neutral stimulus with the administration of an

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emetic dose of morphine. Such classical conditioning processes are felt to be responsible for what is called anticipatory nausea and vomiting in the clinical situation (5,29,38), and the incidence of such emetic responses has been reported to be as high as 44% of the patients receiving oncolytic therapy (5,29). Because antiemetics currently in use are not effective in reducing anticipatory nausea and vomiting in the clinical situation, we felt it important to study the effects of several agents against such conditioned vomiting in the bird. Thus, a group of pigeons was conditioned to vomit in response to placement into the observation chambers, and the antiemetic effects of the 5-HT_{1A} agonists, pentobarbital and a 5-HT_3 antagonist were also evaluated in this model of emesis.

METHOD

Subjects

A pool of 40 male White Carneaux pigeons (Palmetto Pigeon Plant, Sumter, NC) were kept in individual stainless steel cages with water and crushed oyster shells continuously available except during experimental sessions. Six of these birds were used in the conditioning study. Five pigeons that had become incidentally conditioned during the acute phase were also studied to evaluate extinction of the conditioned response. Temperature (22°C ± 1) and humidity in the colony room were kept constant. Pigeons were maintained at 85–90% of their free-feeding body weights by a once daily feeding of 20 g of Purina Pigeon Checkers. All testing was conducted during the illuminated phase of the light/dark cycle (0600–1800 h). If vomiting occurred, the pigeons were given an additional 20 g of feed after they were returned to their home cages at the conclusion of the observation period. Individual subjects were allowed a recovery period of at least 3 days between each drug test.

Procedure

Acute. On a test day, the pigeons first were fed in their home cages. Five minutes later, the birds were injected in the breast muscle (IM) with various doses of the test drugs or saline and returned to their home cages for a 15-min pretreatment period. DTG was then injected (IM) and the pigeons were placed into Plexiglas chambers where they were observed continuously for the next hour. Home cages were examined for the presence of vomitus when the pigeons were removed at the end of the pretreatment period. As 5.6 mg/kg of DTG reliably caused vomiting in 100% of the pigeons during initial testing, this dose was used in all subsequent experiments. The following drugs were tested as blockers of DTG-induced emesis: 8-OH-DPAT hydrobromide (0.02–0.64 mg/kg); LY228729 (0.01–0.32 mg/kg); NAN-190 hydrobromide (0.32–3 mg/kg), tropisetron (0.008–0.5 mg/kg), and pentobarbital sodium (10 mg/kg). In addition, NAN-190 (3 mg/kg) was injected 15 min prior to 8-OH-DPAT in an attempt to antagonize the antiemetic effects of 8-OH-DPAT. As tropisetron induced an emetic response when injected in the absence of DTG, it was also studied in combination with 8-OH-DPAT. All injections were administered into the breast muscle in a volume of 1 ml/kg of body weight, except the 3 mg/kg dose of NAN-190 which was injected in a 2 ml/kg volume because of solubility difficulties.

Chronic. During chronic studies, four birds were injected with 0.64 mg/kg of 8-OH-DPAT daily for 16 days and replaced into their home cages. They were challenged with an injection of 5.6 mg/kg of DTG 15 min after administration of 8-OH-DPAT on days 5, 9, 12, and 16 and then observed as usual.

Conditioned. Conditioning studies were carried out in the same manner as the acute studies, except that greater care was taken to ensure that the pigeons were handled in exactly the same way on each occasion (e.g., same observation chamber, time of day, site of injection, order of testing). During each of the tests, three birds were preinjected with saline and three with the test drug. The order of test sessions was always saline, test drug, and then saline again. Each pigeon's performance under the test drug was compared to the average of the before and after saline sessions. A second group of five pigeons was also deliberately conditioned to examine extinction of the conditioned response. These five birds had shown evidence of incidental conditioning (i.e., retching or vomiting in the pretreatment period) during the acute studies with DTG and were eliminated from that study. The arbitrary criterion for classification as a conditioned vomiting bird for the sake of inclusion in the conditioning studies was a latency for the first emetic response under 4 min in three consecutive training trials.

Data Analysis

The latency for the onset of emesis, the number and time of episodes, and the weight of the pigeons at the end of the 1-h observation period were recorded. An emetic response consisted of either vomiting (the active expulsion of matter from the pigeon's beak) or retching (vomiting movements without the expulsion of either fluids or solids). A quiescent period of at least 30 s was required between episodes before a separate response was scored. When LY228729 was tested in the unconditioned pigeons, only the presence of vomit and weight loss at the end of 1 h were noted. When the pigeons did not vomit during the 1-h observation period, a value of 60 min was entered for the latency and 0 for the number of episodes. These values were used when calculating group means and standard errors. Statistical differences between groups were determined by analysis of variance. Tukey–Kramer HSD was used for comparison of all pairs. A $p < 0.05$ was used to refer to any difference as statistically significant. ED_{50}s and 95% confidence limits were calculated using a method developed by Dr. Kerry Bemis (Eli Lilly & Co.) for use with JMP software.

Drugs

DTG was purchased from Eastman Organic Chemicals (Rochester, NY). 8-OH-DPAT hydrobromide and NAN-190 hydrobromide were purchased from Research Biochemicals (Natick, MA). Tropisetron (ICS-205 930) and LY228729 were synthesized by Eli Lilly and Co. (Indianapolis, IN). Pentobarbital sodium was purchased from Sigma Chemical Co. (St. Louis, MO). DTG was dissolved in 0.1 ml of 10% lactic acid solution per 5 ml of final solution and then brought to a volume of 5.6 mg/ml by the addition of sterile distilled water. NAN-190 was dissolved in sterile distilled water with the addition of a few drops of 10% lactic acid, and then gently warmed and sonicated. LY228729 was dissolved in sterile water with the addition of a drop of lactic acid. Tropisetron, pentobarbital and 8-OH-DPAT were dissolved in saline.

RESULTS

Acute Studies

DTG induced a dose-related emetic response (Table 1). A dose of 5.6 mg/kg DTG reliably caused vomiting in 100% of the pigeons tested, with a mean latency of 22.6 min (±4 SEM)

TABLE 1

THE EMETIC EFFECTS OF DTG IN THE PIGEON

Dose of DTG (mg/kg)	Vomited/Tested	Number of Episodes	Latency to Onset (min)	Weight Loss (g)
1.4	0/6	0	60	8 (±1)
2.8	5/7	1 (±1)	43 (±7)	18 (±4)
5.6	7/7	5 (±1)	22.6 (±4)	28 (±4)

The data are presented as means (± SEM). The number of episodes is the combined number of vomits and retches. A latency of 60 min indicates a test with no emetic response. The weight loss is the amount of weight lost by the pigeons from the time of pretreatment until the end of the 1 h observation period.

and an average of 5 (± 1 SEM) episodes of regurgitation. Only two incidents of retching in the absence of vomiting were noted at the 5.6 mg/kg dose of DTG. At the 5.6 mg/kg dose, the average weight lost during the 1-h observation period was 28 g (± 4 SEM), and emesis was evident throughout the entire 1-h observation period. All pigeons continued to vomit when challenged with DTG once or twice a week over a period of 2–3 months.

With the exception of tropisetron, none of the drugs given in combination with saline (in the absence of DTG) produced emesis. Saline, administered to unconditioned pigeons before DTG, did not modify the time of onset, the number of episodes or the weight loss induced by 5.6 mg/kg DTG in the initial dose-response curve. However, when challenged with only saline before being placed into the observation box, 41% of the pigeons (14 birds) used in the acute studies showed an incidentally conditioned emetic response after an average of 8.8 (± 0.99 SEM) trials. As this was an unintentionally conditioned emetic response, such data and the immediately preceding test data from the same pigeons were eliminated from analysis. Other pigeons failed to develop a conditioned response even after an average of 15.5 (± 0.71 SEM) trials when they were eliminated from the study.

Pretreatment with the 5-HT_{1A} agonists 8-OH-DPAT and LY228729 produced a dose-related attenuation of the DTG-induced emetic response (Fig. 1) with ED_{50}s (95% confidence interval) of 0.075 (0.056–0.105) and 0.04 (0.031–0.052) mg/kg, respectively. Vomiting was completely blocked by 0.64 mg/kg of 8-OH-DPAT and by 0.16 mg/kg of LY228729. The lowest dose of both compounds (0.02 and 0.01 mg/kg, respectively) did not protect the pigeons from vomiting.

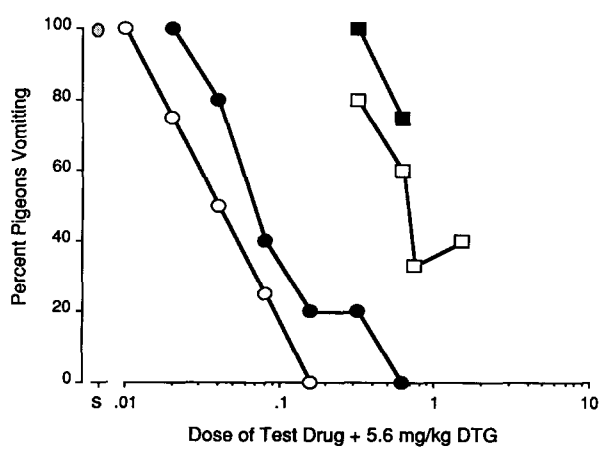

FIG. 1. The effects of 8-OH-DPAT (closed circles ●), LY228729 (open circles ○), NAN-190 (open squares □), NAN-190 (3 mg/kg) + 8-OH-DPAT (closed squares ■) and saline (unconnected circle) on vomiting induced by injection of 5.6 mg/kg of DTG.

NAN-190 had no effect when administered in the absence of DTG. However, doses of NAN-190 from 0.3 to 3 mg/kg decreased the percentage of pigeons that vomited in response to DTG, with an ED_{50} (95% confidence limits) of 0.75 mg/kg (0.011–0.607) (Fig. 1 and Table 2). When injected 15 min prior to 8-OH-DPAT, 3 mg/kg of NAN-190 blocked the protective effects of 8-OH-DPAT (Table 2). The effectiveness of 0.64 mg/kg of 8-OH-DPAT was reduced from 100% to 25% and that of 0.32 mg/kg from 80% to 0% of the pigeons protected from vomiting.

In contrast to the 5-HT_{1A} compounds, the 5-HT_3 antagonist tropisetron was ineffective in preventing DTG-induced emesis, with only a slight reduction in vomiting occurring at the 0.016 mg/kg dose (Table 3). The 0.5 mg/kg dose of tropisetron always produced vomiting in the absence of DTG (data not shown). The emetic effects induced by 0.5 mg/kg of tropisetron (6.5 ± 0.76 episodes with an average latency of 7.1 min) were abolished by an injection of 0.64 mg/kg of 8-OH-DPAT (no emetic episodes in four birds observed for 1 h) administered 15 min prior to tropisetron.

Chronic Study

8-OH-DPAT effectively continued to antagonize the effects of DTG even during chronic administration (data not shown). Four of the five pigeons tested remained completely protected during the 16-day period of daily administration of 8-OH-DPAT. Two episodes of vomiting were noted in one pigeon near the end of the one hour observation period during the second challenge (9th day of 8-OH-DPAT administration) with DTG. This pigeon did not vomit during subsequent challenges on day 12 or 16 of 8-OH-DPAT administration.

Conditioned Studies

Of the five pigeons originally selected to be conditioned, two were not used because they failed to develop a consistent response latency, although they did have an emetic response when tested with saline in the absence of DTG. Three birds who showed evidence of incidental conditioning were added to the group and trained to criterion, bringing the total number of pigeons conditioned to vomit to six (the primary conditioned group). The average latency to vomit on first exposure to DTG was 18.3 (± 2.2 SEM) min in this group of birds and was not significantly different than the average latency to

TABLE 2

COMPARISON OF THE EFFECTS OF 8-OH-DPAT AND NAN-190 ON DTG-INDUCED EMESIS

Pretreatment	Vomited/Tested	Latency to Onset (min)	Number of Episodes	Weight Loss (g)
Saline	14/14	18.4 (±2.3)	6.1 (±0.4)	23 (±2)
8-OH-DPAT				
0.02 mg/kg	5/5	15.5 (±2.5)	5 (±0.7)	23 (±4)
0.04 mg/kg	4/5	34.6 (±9.8)	1.8 (±0.7)	11 (±4)
0.08 mg/kg	2/5	43.6 (±10.3)*	1.3 (±0.9)*	10 (±4)
0.16 mg/kg	1/5	53.9 (±6.2)*	0.2 (±0.2)*	4 (±1)*
0.32 mg/kg	1/5	50.27 (±9.7)*	0.4 (±0.4)*	4 (±2)*
0.64 mg/kg	0/5	60*	0*	4 (±1)*
NAN-190				
0.3 mg/kg	4/5	31.7 (±8)	5.2 (±2)	31 (±8)
0.75 mg/kg	3/5	37.4 (±9)	3 (±1)	4 (±2)*
1.5 mg/kg	2/6	53.7 (±5)*	0.67 (±0)*	8 (±2)*
3 mg/kg	2/5	48.6 (±7)*	1.2 (±1)*	3 (±2)*
NAN-190 (3 mg/kg) + 8-OH-DPAT				
0.32 mg/kg	4/4	10.7 (±2.1)†	7.2 (±2.2)†	7.3 (±2)
0.64 mg/kg	3/4	24.9 (±12)†	2.5 (±1)	13.5 (±4)†

The data represent means (± SEM).

*Significantly different than saline + DTG control; †Significantly different than either 0.32 mg/kg or 0.64 mg/kg 8-OH-DPAT + DTG (Tukey-Kramer $p < 0.05$).

vomit found in unconditioned birds. The birds reached the arbitrary conditioning criterion of three consecutive trials with a latency below 4 min in an average of 22 (±4 SEM) trials. The actual latency decreased over trials to an average of 1.05 min (±0.26 SEM) during the last three training trials and did not vary significantly from this value during the saline control tests.

Figure 2 shows the pattern of emetic responses in both conditioned and unconditioned birds over the 1-h observation period. Pigeons not conditioned do not vomit or retch during the initial 5-min period after DTG administration. On only three occasions did pigeons begin to vomit before 10 min had elapsed (at 7.5, 7.8, and 9.2 min). In contrast, all of the conditioned birds consistently began to vomit within the first 5 min and continued to do so throughout the entire observation period. Consequently, the total number of emetic episodes in the 1-h observation period increased from 6.1 (±0.4 SEM) in the unconditioned saline controls to an average of 10.2 (±0.5 SEM) episodes in the conditioned saline control birds.

Administration of tropisetron (0.016, 0.064, and 0.128 mg/kg) did not modify the emetic response in the conditioned birds at any of the doses tested. The pattern for the 0.064 mg/kg dose is shown in Fig. 2. The effects of pentobarbital were dependent on whether or not the subjects exhibited conditioned emesis (Table 4). Administration of 10 mg/kg of pentobarbital 15 min prior to DTG in the conditioned pigeons resulted in a significantly increased latency to the onset of emesis and a significantly decreased number of emetic episodes ($p < 0.05$) compared to the performance of the same pigeons when pretreated with saline. Injection of the same dose of pentobarbital to unconditioned pigeons, however, had no effect upon either the latency or the number of emetic episodes compared

TABLE 3

EFFECTS OF TROPISETRON ON DTG-INDUCED EMESIS (ACUTE STUDY)

Pretreatment + DTG (5.6 mg/kg)	Vomited/Tested	Latency to Onset (min)	Number of Episodes	Weight Loss (g)
Saline	14/14	18.4 (±2.3)	6.1 (±0.4)	23 (± 2.4)
Tropisetron				
0.008 mg/kg	5/5	19.2 (±4.3)	5.2 (±5.8)	11 (±4.2)
0.016 mg/kg	3/5	38.4 (±9.1)	2.6 (±1.3)	11 (±7)
0.032 mg/kg	5/5	29 (±3.2)	4 (±1)	12 (±4)
0.064 mg/kg	5/5	21.1 (±8.4)	4.8 (±2.2)	17 (±7)
0.125 mg/kg	5/5	12.7 (±2.9)	4.8 (±1.2)	24 (±5)

The data are means (±SEM).

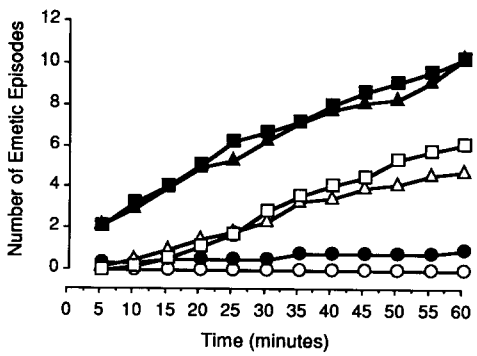

FIG. 2. The number of emetic episodes occurring in consecutive 5-min periods after the injection of 5.6 mg/kg of DTG. The unconditioned control (open squares □) is the average emetic response of six birds over the first three trials in which saline was administered 15 min prior to the DTG. The conditioned control (closed squares ●) is the average response to all the saline control injections given to all conditioned pigeons across all the drug trials. Standard error bars for both the conditioned and uncondtioned controls are obscured by the symbols. The 5-HT_{1A} selective drugs, 8-OH-DPAT (0.64 mg/kg) (closed circles ●) and LY228729 (0.32 mg/kg) (open circles ○), blocked both the conditioned and the unconditioned emetic response. Pentobarbital (10 mg/kg) (open triangle △) blocked the condtioned, but not the unconditioned response. The 5-HT_3 selective agent, tropisetron (0.064 mg/kg) (closed triangle ▲), did not attenuate either the conditioned or the unconditioned emetic response.

to unconditioned birds pretreated with saline. In fact, the pattern of the emetic responses of the conditioned pigeons pretreated with pentobarbital is very similar to that of unconditioned subjects pretreated with saline (Fig. 2). In contrast to tropisetron and pentobarbital, both LY228729 (0.32 mg/kg) and 8-OH-DPAT (0.64 mg/kg) were effective in preventing retching and vomiting in the conditioned birds (Fig. 2) as well as in the unconditioned pigeons.

A second group of five pigeons that had shown incidental conditioning during the acute phase were also trained to the same criterion as the primary conditioned group to determine the number of unreinforced trials (no DTG) that would be required to extinguish the conditioned emetic response. The average latency for the first emetic response of the second group on the last three training trials was 0.8 (±0.23 SEM) min, which was not significantly different than that of the original conditioned group's last three training trials. On the first extinction trial (no administration of DTG), all of the birds vomited. The average latency to the first emetic response was 0.95 (±0.4 SEM) min which was not significantly different than the latency prior to the beginning of extinction. However, because there was no unconditioned elicitation of vomiting from the DTG, the average number of episodes decreased significantly ($p < 0.05$) from 8.8 (±0.8 SEM) on the last training trial to 3.2 (±1.2 SEM) on the first extinction trial (Table 5). The conditioned emetic response extinguished in an average of 3 (±0.3) trials.

DISCUSSION

The present study confirmed that injection of 5.6 mg/kg of DTG produces a robust emetic response in pigeons (19). As all the pigeons appeared to be recovered by the next day and did not develop tolerance to DTG (20), complete dose–response curves may be obtained using relatively few animals. This represents a significant advantage over some of the other emetic stimuli that have been tested in the pigeon but which produce a much lower percentage of vomiting birds (23), or which produce 100% responding but are damaging to the animals such that they can only be used once [e.g., cisplatin, (34)].

Approximately 41% of the pigeons used in the acute studies eventually began to vomit when they were injected with vehicle in the absence of DTG and placed in the observation box. Therefore, all of the birds were eliminated from the acute studies after approximately 15 trials because of the possibility of an unconditioned emetic response interfering with the acute test results. A similar phenomenon has been reported in humans in that some patients receiving intravenous emetogenic chemotherapy experience nausea and vomiting prior to their infusion. Estimates of the prevalence of this response vary widely [e.g., 23% (41); 35% (5); 41% (8); 18–44% (29)]. Although anxiety appears to play a role in the etiology of anticipatory nausea (4), the response most likely develops as a result of classical conditioning (9,10,21,31). According to this paradigm, one or more distinctive features of the clinic (visual, olfactory, etc.) function as the conditioned stimulus (CS). The unconditioned stimulus (UCS) is the emetogenic chemotherapy infusion and the unconditioned response (UCR) is the nausea and vomiting that frequently occur 1–2 h postinjection. An anticipatory response may occur after one to a few chemotherapy cycles and can be difficult to treat (41). In the present study, the CS is the environmental cue of being injected and placed into the observation box. The UCS is the injection of DTG which is followed by retching and vomiting, the UCR. After a few trials, the latency to the onset of the

TABLE 4

THE EFFECTS OF PENTOBARBITAL ON CONDITIONED AND UNCONDITIONED DTG-INDUCED EMESIS

Pretreatment	Tested/Vomited	Conditioned to Vomit	Latency (min)	Episodes
Saline	6/6	yes	2.6 (±0.6)	8.5 (±0.5)
Pentobarbital	6/6	yes	17.6 (±5.2)*	4.8 (±0.9)*
Saline	14/14	no	18.4 (±2.3)	6.1 (±0.4)
Pentobarbital	4/4	no	13.8 (±8)	5.7 (±1.8)

The data are means (±SEM).
*Significantly different than the saline + DTG conditioned control group ($p < 0.05$).

TABLE 5

COMPARISON OF THE LAST THREE TRAINING TRIALS WITH EXTINCTION TRIALS IN THE SECOND GROUP OF PIGEONS CONDITIONED TO VOMIT

Trial	Latency	Episodes
Last three training trials	0.8 (±0.23)	8.8 (±0.8)
First extinction trial	0.95 (±0.4)	3.2 (±1.2)*
Second extinction trial	14.2 (±11.7)	2 (±1.1)
Third extinction trial	37.7 (±13.8)	0.5 (±1.2)

Comparison of the latency in minutes to the onset of either retching or vomiting and the number of emetic episodes during the last three training trials and the first three extinction trials in the second group of conditioned birds.
*Significantly decreased ($p < 0.05$).

emetic response decreased dramatically, indicating the presence of a conditioned response (CR) in some animals. Also, the number of emetic episodes greatly increased, suggesting that one type of emetic response (CR) had been superimposed upon another (UCR). Once the response latency stabilized in the intentionally conditioned pigeons, all of these animals vomited when placed in the observation box after vehicle administration, but with a significantly decreased number of episodes. The fact that this response extinguished after a few nonreinforced trials (no DTG injection) also supports the conclusion that this is a conditioned response.

During the drug tests in the primary conditioned group, DTG was always administered to avoid the possibility of confounding the results by possibly extinguishing the conditioned emetic response. The latency and increased number of episodes of retching and vomiting were used as indices of conditioning by comparing saline + DTG before and after the test drug + DTG trials in the same subjects. Pentobarbital significantly increased the latency and significantly decreased the number of emetic responses in the conditioned birds. In fact, the response of conditioned pigeons under pentobarbital was indistinguishable from that of unconditioned pigeons given pentobarbital and then DTG, suggesting that the conditioned emetic response, but not the unconditioned emetic response, was blocked. This may occur as a result of the anxiety-reducing properties of pentobarbital in pigeons because a similar dose will also increase rates of punished responding, a measure of antianxiety activity [e.g., (42)]. Anxiety may contribute to the severity of anticipatory nausea in chemotherapy patients (4) and various behavioral anxiety-reducing strategies have proved useful in controlling anticipatory nausea and vomiting (10). In the present study, it is not possible to distinguish motor effects on emesis from the antianxiety properties of the 5-HT_1A agonists in the blocking of the conditioned emetic response because 5-HT_1A agonists produce robust antianxiety effects on punished responding in pigeons [e.g., (1,14)].

Both 8-OH-DPAT and LY228729, potent and selective 5-HT_{1A} agonists, abolished conditioned, as well as the DTG-induced, unconditioned emesis. The conclusion that the antiemetic effect is through the 5-HT_{1A} receptor subtype is supported by the fact that the putative 5-HT_{1A} antagonist, NAN-190, which has a high affinity for the 5-HT_{1A} site (16), attenuated the antiemetic effects of 8-OH-DPAT. In the pigeon, NAN-190 blocks both the discriminative and anticonflict effects of 8-OH-DPAT (1,6) without influencing either response when administered by itself. However, as NAN-190 also produced an antiemetic response when administered by itself, the results of the present study are in agreement with work in other species in which NAN-190 is a partial agonist. For instance, Przegalinski et al. (35) found that NAN-190 blocked the behavioral syndrome but not the hypothermia and hormonal response induced by 8-OH-DPAT. Both hypothermia and increased serum corticosterone levels, similar to those seen with other 5-HT_{1A} partial and full agonists, were produced by NAN-190 when given alone.

Administration of 8-OH-DPAT over a period of 16 days did not substantially reduce its antiemetic effects. Other workers have found that tolerance to some of the effects of 8-OH-DPAT develop over a relatively short period of time in the mouse [e.g., (13)] and the rat [e.g., (33)]. Little information is available on the development of tolerance to the chronic administration of 5-HT_{1A} agonists in the pigeon. However, Barrett and co-workers (30) found that tolerance to the reduction in the serotonin metabolite 5-HIAA, induced by acute injections of 8-OH-DPAT, did not occur in the pigeon during the chronic administration of 8-OH-DPAT.

In contrast to the 5-HT_{1A} agonists, 5-HT_3 antagonists do not exhibit a wide spectrum antiemetic profile. Although tropisetron, a 5-HT_3 selective antagonist, is a potent compound in the prevention of cisplatin-induced emesis in the ferret (11), it was not an effective antiemetic in the present study, suggesting that activity at the 5-HT_3 receptor is not involved in either the conditioned or DTG-induced emesis. Selectively blocking 5-HT_3 binding sites inhibits radiation and oncolytic-induced emesis (3) but is ineffective against a range of other emetic stimuli such as either xylazine-induced vomiting or motion sickness in cats (24), various chemical emetogens in shrews (39), or apomorphine-evoked emesis in ferrets (28). Tropisetron was not only ineffective in blocking the emetic effects of DTG, but high doses induced rapid onset vomiting in the absence of the DTG stimulus that could be blocked by administration of 0.64 mg/kg 8-OH-DPAT. Other workers [e.g., (22,27,34,37)] have also found that some 5-HT_3 antagonists paradoxically both inhibit cisplatin-induced vomiting and display intrinsic emetic activity. Middlefell et al. (27) has suggested that such emetic responses by another HT3 antagonist, zacopride, could be attributed to weak partial agonist activities at 5-HT_3 receptors.

5-HT_{1A} agonists block emesis in cats evoked by motion, drugs (xylazine), and cytotoxic agents such as cisplatin (26). Each of these stimuli exerts its effect through a different mechanism. Cisplatin-induced emesis may be mediated either peripherally through vagal afferents or centrally (3), whereas xylazine-induced emesis is mediated by the chemoreceptor trigger zone in the area postrema (17), and motion sickness is mediated through the vestibular system. In the present study, 5-HT_{1A} agonists blocked both a chemically induced and a conditioned emetic response. Because of the variety of pathways involved in these responses, the antiemetic actions of 5-HT_{1A} agonists may be through interference with the integrative mechanisms for emesis or blocking the efferent pathway.

In summary, these observations replicate the findings that DTG (5.6 mg/kg) reliably produces a robust emetic response in pigeons. The present data extend the antiemetic effects of agonists at the 5-HT_{1A} receptor subtype to the pigeon and different emetic stimuli, including DTG and the conditioned stimuli that occasion nausea and vomiting in the anticipatory nausea and vomiting situation, suggesting that these agonists will have broad spectrum antiemetic effects.

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