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Peptide YY_{3-36} and 5-Hydroxytryptamine Mediate Emesis Induction by Trichothecene Deoxynivalenol (Vomitoxin)

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Deoxynivalenol (DON, vomitoxin), a trichothecene mycotoxin produced by *Fusarium* sp. that frequently occurs in cereal grains, has been associated with human and animal food poisoning. Although a common hallmark of DON-induced toxicity is the rapid onset of emesis, the mechanisms for this adverse effect are not fully understood. Recently, our laboratory has demonstrated that the mink (*Neovison vison*) is a suitable small animal model for investigating trichothecene-induced emesis. The goal of this study was to use this model to determine the roles of two gut satiety hormones, peptide YY_{3-36} (PYY_{3-36}) and cholecystokinin (CCK), and the neurotransmitter 5-hydroxytryptamine (5-HT) in DON-induced emesis. Following ip exposure to DON at 0.1 and 0.25 mg/kg bw, emesis induction ensued within 15–30 min and then persisted up to 120 min. Plasma DON measurement revealed that this emesis period correlated with the rapid distribution and clearance of the toxin. Significant elevations in both plasma PYY_{3-36} (30–60 min) and 5-HT (60 min) but not CCK were observed during emesis. Pretreatment with the neuropeptide Y2 receptor antagonist JNJ-31020028 attenuated DON- and PYY-induced emesis, whereas the CCK1 receptor antagonist devezapide did not alter DON's emetic effects. The $5\text{-}HT_3$ receptor antagonist granisetron completely suppressed induction of vomiting by DON and the 5-HT inducer cisplatin. Granisetron pretreatment also partially blocked PYY_{3-36}-induced emesis, suggesting a potential upstream role for this gut satiety hormone in 5-HT release. Taken together, the results suggest that both PYY_{3-36} and 5-HT play contributory roles in DON-induced emesis.

Key Words: **mycotoxin; trichothecene; deoxynivalenol; vomitoxin; 5-hydroxytryptamine; peptide YY_{3-36}; emesis.**

Deoxynivalenol (DON), a trichothecene mycotoxin produced by *Fusarium* sp. that frequently contaminates cereal staples, has been documented to cause human and animal food poisoning (Canady *et al.*, 2001; Pestka, 2010a). DON remains stable during cereal storage and is relatively resistant to food processing techniques enabling it to persist in foods consumed by humans and domestic animals (Jackson and Bullerman, 1999). There is a growing concern that global changes in agricultural practice and climate have increased the incidence of *Fusarium* head blight with the end result being rising levels of DON contamination in grain-based foods (Pestka, 2010b).

DON was originally called "vomitoxin" by United States Department of Agriculture (USDA) researchers because of its potent emetic effects in pigs (Vesonder *et al.*, 1973). Consistent with this colloquial name, investigations of foodborne illnesses associated with *Fusarium* and/or DON contamination identified vomiting as one of the primary symptoms (Luo, 1994; Ueno, 1987; Yoshizawa, 1983). For example, between 1961 and 1991, 53 gastroenteritis outbreaks in China were etiologically linked to consumption of cereals containing *Fusarium* and/or DON, with the largest outbreak affecting over 130,000 people (Luo, 1994). DON is not routinely measured in foods or clinical samples associated with U.S. food poisoning outbreaks by public health agencies as is commonly done for pathogens and bacterial toxins. However, in one notable exception, DON was sought and detected at < 1 ppm in the absence of other putative food poisoning agents in burritos associated with 16 large outbreaks of gastroenteritis with a characteristic rapid onset vomiting that affected over 1,900 schoolchildren in seven states (Anonymous, 1999; Steinberg *et al.*, 2006).

Despite emesis being a likely consequence of DON-related food poisoning, relatively few studies have focused on the underlying mechanisms of this effect or potential human sensitivity. Emesis, a reflex that forcefully drives out contents of the upper gastrointestinal (GI) tract through the oral cavity (Andrews and Hawthorn, 1988), serves as a protective mechanism against food poisoning. Severe emesis can adversely affect human and animal health by causing nausea, disrupting normal nutrition, hydration, and electrolyte balance.

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The emetic response is a highly complex process integrating neurotransmitters, hormones, and visceral afferent neurons that are coordinated by a neuronal network known as the central pattern generator (CPG) (Hornby, 2001; Koga and Fukuda, 1992; Miller, 1999). The CPG is located in the medulla oblongata of the hindbrain where it coordinates the efferent autonomic and motor neurons.

Cytotoxic drugs used in the treatment of cancer trigger vomiting in at least two ways. One mechanism involves the action of peripheral blood- and cerebrospinal fluid (CSF)–borne emetic stimuli (e.g., hormones and neurotransmitters) at the area postrema (AP) of the medulla. The AP is a circumventricular organ that lies between the brain parenchyma and the CSF-containing ventricles, and it is believed to be the primary chemoreceptor trigger zone for humoral agent–mediated emesis (Carpenter, 1990). The AP lacks a specific blood-brain diffusion barrier allowing it to sense emetic stimuli in both the blood and CSF, leading to activation of the CPG and subsequent emesis (Borison, 1989; Carpenter, 1990; Hornby, 2001). In a second possible mechanism, emetic stimuli may act on the enterochromaffin (EC) cells to induce a local release of emetic mediators such as 5-HT. These mediators trigger emesis by binding to the corresponding receptors located on vagal afferent terminals that relay emetic stimuli to nucleus tractus solitarius (NTS) and ultimately activate the CPG (Andrews *et al.*, 1990; Andrews and Horn, 2006; Hornby, 2001). The monoamine 5-hydroxytryptamine (5-HT, serotonin) is a well-known mediator of emesis, nausea, appetite, and GI functions (Endo *et al.*, 2000; Kucharczyk and Harding, 1990; Stables *et al.*, 1987). Although most 5-HT is synthesized and released from EC cells in the GI tract, it can also be produced in the neurons of the CNS (Kim and Camilleri, 2000). Receptors for this amine are the major targets of antiemetic drugs that combat the emetic actions of chemotherapeutic agents such as cisplatin (Percie du Sert *et al.*, 2011). Although it could not be demonstrated in the pig that DON upregulates plasma concentrations of 5-HT or its metabolite 5-hydroxyindoleacetic acid (5-HIAA) (Prelusky, 1994), 5-HT_3 receptor antagonists prevent DON-induced vomiting in this species (Prelusky and Trenholm, 1993). Thus, DON's emetic effects likely involve serotonergic pathways.

The gut satiety hormones peptide YY (PYY) and cholecystokinin (CCK) are released in mice upon DON exposure (Flannery *et al.*, 2012) and might also have the potential to mediate DON-induced emesis. PYY is a 36-amino-acid protein of the pancreatic polypeptide hormone family expressed in both endocrine cells and neurons (Ekblad and Sundler, 2002). PYY is released by L-endocrine cells of the ileum and colon in two endogenous forms, $PYY_{1–36}$ and $PYY_{3–36}$, with the latter exhibiting greater biological activity and abundance in the circulation (Pittner *et al.*, 2004). $PYY_{3–36}$ is an agonist of the neuropeptide Y2 receptor (Y2R) (Sloth *et al.*, 2007) functioning to induce satiety and decrease gastric emptying (Ballantyne, 2006; Batterham *et al.*, 2002; Halatchev and Cone, 2005; Koegler *et al.*, 2005). PYY regulates food intake by acting on peripheral Y2Rs located on vagal afferent neurons (Abbott *et al.*, 2005) and by acting centrally via the NTS and hypothalamus (Blevins *et al.*, 2008). Importantly, PYY is highly emetogenic in dogs, a response that is mediated by the AP (Harding and McDonald, 1989; Perry *et al.*, 1994). Furthermore, both nausea and vomiting are adverse side effects of PYY administration in humans (Gantz *et al.*, 2007; Sloth *et al.*, 2007).

In the mouse, ip and oralingual exposure to DON-induced rapid (15 min) increases in plasma PYY (Flannery *et al.*, 2012). It was further shown that pretreatment with an Y2R antagonist suppressed DON-induced anorexia, implicating a role for PYY. Although mice are useful for studying food intake, they are incapable of vomiting and therefore not an effective model for discerning linkages between DON-induced PYY and emesis. Nevertheless, the rapid and transient nature of $PYY_{3–36}$-induced emesis (Harding and McDonald, 1989) is remarkably similar to that previously observed with DON in studies of the pig and other animal species (Forsyth *et al.*, 1977; Hughes *et al.*, 1999; Pestka *et al.*, 1987; Wu *et al.*, 2013), suggesting that this gut satiety hormone could be an unrecognized factor in DON-induced nausea and vomiting.

CCK is produced by I-cells in the duodenum (Liou *et al.*, 2011) and acts primarily on peripheral CCK1 receptors (CCK1Rs) located on intestinal vagal afferents or located within the dorsal vagal complex (Baptista *et al.*, 2007; Kopin *et al.*, 1999; Sullivan *et al.*, 2007). Previous research in monkeys has demonstrated nausea and vomiting upon iv treatment with CCK (Perera *et al.*, 1993). Furthermore, DON markedly elevates plasma CCK concentrations in the mouse along with PYY (Flannery *et al.*, 2012). It could thus be speculated that CCK might also contribute to nausea and vomiting following DON exposure.

Critical questions remain regarding the precise roles of 5-HT and the aforementioned gut satiety hormones in DON-induced emesis. Recently, our lab developed a mink model to study trichothecene-induced emesis and employed it to compare emetic effects between DON and its congeners (Wu *et al.*, 2013). The sensitivity of this species to DON-induced emesis was found to be similar to that observed in larger animals. Here, we utilized the mink to test the hypothesis that 5-HT, $PYY_{3–36}$, and CCK coordinate DON-induced emesis. The results indicated that plasma 5-HT and $PYY_{3–36}$ but not CCK were elevated during DON-induced emesis, and furthermore, chemical antagonists for 5-HT and PYY receptors but not CCK receptor suppressed induction of vomiting by this mycotoxin.

MATERIALS AND METHODS

Laboratory animals. Animal treatment followed National Institutes of Health guidelines and were approved by the Michigan State University Institutional Animal Care and Use Committee. Sixty standard dark, female mink (*Neovison vison*) of 1–2 years of age (average weight = 1.2 ± 0.2 kg) were obtained from Michigan State University (MSU) Experimental Fur Farm. Animals were housed singly in wire cages (62 cm long × 25 cm wide × 38 cm high) within an open-sided pole barn and were provided with a nest

box (24 cm long × 24 cm wide × 29 cm high) with aspen shavings and excelsior. Temperature, humidity, and photoperiod were dependent on ambient environment. Experiments were conducted in October, November, and December 2011 and January 2012, a period in which the mink were not in the estrus state, with average temperatures being 41–60, 32–47, 22–34, and 17–30°F for these months, respectively. These housing conditions met those specified in the Standard Guidelines for the Operation of Mink Farms in the United States (Fur Commission USA, 2010). Mink were acclimated for a minimum of 1 week prior to the initial experiment and fed the MSU Experimental Fur Farm ranch diet, which is formulated to meet the nutrient requirements of mink. To minimize variation in gastric contents among test animals, mink were fasted (no feed but water available *ad libitum*) for 24 h (day 1) prior to all experiments and then given 50 g feed just prior to the treatment. To minimize the number of animals used, mink employed for receptor antagonist studies were given a minimum 2 weeks of washout period between experiments, except cisplatin-treated mink (positive control to demonstrate that granisetron is an effective antiemetic in mink as in other species in response to a well-established emetic stimulus) that were not reused. We based this recovery time on (1) rapid clearance of DON in monogastric species (Pestka, 2010a), (2) rapid reversal of anorectic effects in DON-treated mice (Flannery *et al.*, 2011), (3) rapid clearance of granisetron (Clarke *et al.*, 1994) and JNJ-31020028 (Shoblock *et al.*, 2010) in experimental animals, and (4) preliminary studies with mink showing the absence of anorectic, emetic, or weight effects after 24 h.

Toxin and drugs. Intraperitoneal and subcutaneous injections of the toxin, hormones, or pharmacologic agents were delivered in 1 ml/kg bw using a sterile 20-G, 2.54-cm needle. Delivery volumes were adjusted in accordance with each animal's body weight which was typically between 1.0 and 1.5 kg. Although both ip and po DON exposure can induce emesis in mink, ip administration was employed for all studies to minimize stress and avoid variation in delivery amounts that might occur during oral gavage. DON was obtained from Dr. Tony Durst (University of Ottawa) and purity (> 98%) verified by elemental analysis. For exposure studies, DON was dissolved in filter-sterilized PBS (Sigma-Aldrich, St Louis, MO) and administered to mink at 0.1 and 0.25 mg/kg bw. These doses were previously shown to effectively induce emesis in mink (Wu *et al.*, 2013). Both PYY_{3-36} (Tocris Biosciences, Ellisville, MO) and CCK (23–33, sulfonated; Sigma-Aldrich) were prepared in PBS to provide ip injection at doses up to 0.01 and 0.025 mg/kg bw, respectively. The Y2 receptor antagonist JNJ-31020028, a gift from Dr. P. Bonaventure (Janssen Research & Development, LLC, San Diego, CA), was dissolved in a vehicle composed of Pharmasolve (ISP Technologies, Wayne, NJ) plus 20% 2-hydroxypropyl-β-cyclodextrin (Sigma-Aldrich) and administered by sc injection at a dose of 15 mg/kg bw (Shoblock *et al.*, 2010). The CCK1R antagonist devazepide was dissolved in PBS containing 1% dimethylsulfoxide and administered by sc injection at 0.1 mg/kg bw (Eberle-Wang and Simansky, 1992). Based on prior mink and ferret studies (Qian *et al.*, 2009, 2010a,b; Percie du Sert *et al.*, 2011), the $5\text{-}HT_3$ receptor antagonist granisetron and cisplatin (Tocris) were prepared in PBS and administered by ip injection to mink at doses up to 2.5 and 7.5 mg/kg bw, respectively.

Experimental design. To relate dose-response and kinetics of DON-induced emesis to timing of PYY_{3-36}, CCK, and 5-HT release, 48 fasted mink (n = 4 per group) were given 50 g feed at 8:30 h on the day of dosing and then allowed to eat for 30 min (Fig. 1A). At 9:00 h, 16 mink were dosed by ip injection with PBS, DON (0.1 mg/kg bw), or DON (0.25 mg/kg bw). Each individual retch or vomit was counted as described previously (Wu *et al.*, 2013) and combined to yield total emetic events. Vomiting is characterized as rhythmic abdominal contraction with oral expulsion of either solid or liquid material, whereas retching is defined to responses that mimicked vomiting but without any material being expelled. The distribution of emetic events was calculated based on the total emetic events in 120 min. After 15-, 30-, 60-, and 120-min intervals, mink groups (n = 4 per group) were anesthetized by intramuscular injection with ketamine (30 mg/kg bw) and xylazine (1 mg/kg bw). Blood was collected by heart puncture into vacutainers containing EDTA as anticoagulant, and mink were immediately euthanized by CO_2 exposure.

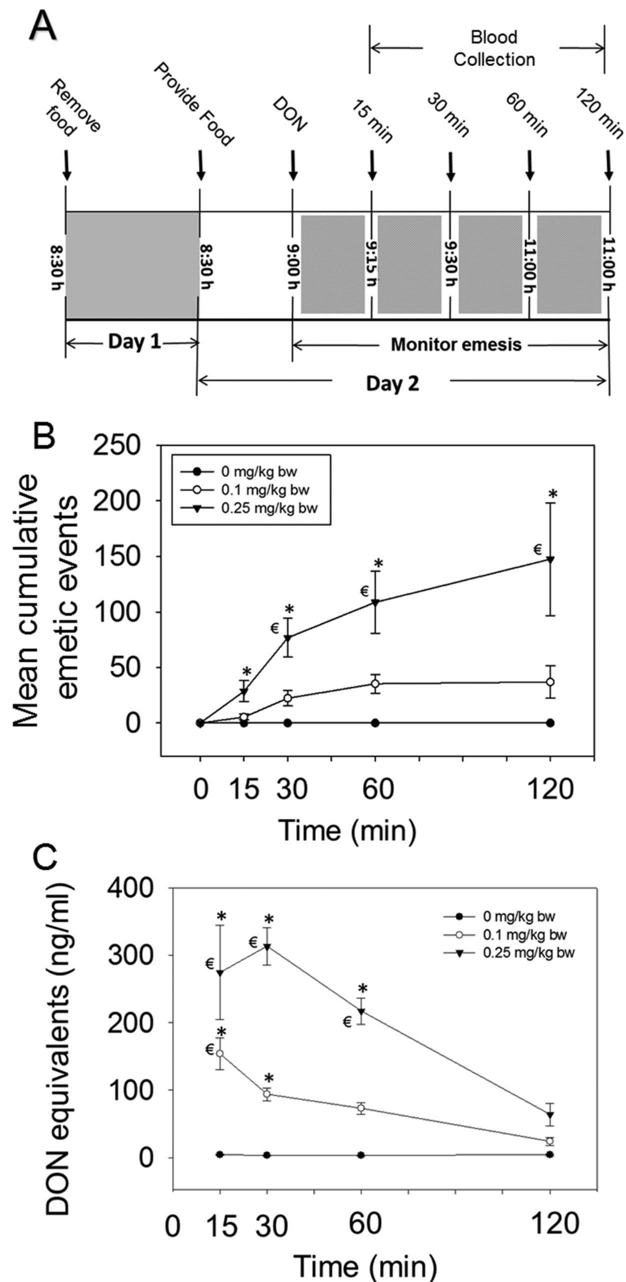

FIG. 1. Emesis induction in mink corresponds to elevation in plasma DON. (A) Experimental design for DON-induced plasma PYY_{3-36} and 5-HT release; (B) mean cumulative emetic events (total retches and vomits) in mink following ip exposure to DON. Data are averages for both responders and nonresponders. The numbers of animals responding/tested at 15, 30, 60, and 120 min were 6/16, 9/12, 8/8, and 4/4, respectively, for 0.1 mg/kg bw DON group and 11/16, 12/12, 8/8, and 4/4, respectively, for 0.25 mg/kg bw DON group. The mean latency time to onset of emesis for the 0.1 and 0.25 mg/kg DON groups were 17 ± 2 and 12 ± 2 min, respectively. (C) Kinetics of plasma DON concentration. Data represent mean ± SEM (n = 4 per group). A two-way ANOVA using Bonferroni t-test was used to assess significant differences in cumulative emetic events and kinetics of DON concentration in plasma. $*p < 0.05$ indicates statistically significant differences in emetic events or DON concentration compared with the control. €$p < 0.05$ indicates a statistically significant difference in emetic events relative to the 0-min time point or significant difference in DON concentration relative to the 120-min time point within a given dose.

Blood was centrifuged at 1000 × g for 10 min. Resultant plasma, which was platelet free, was frozen at −80°C until subsequent analyses for DON, PYY3-36, CCK, and 5-HT by ELISA.

To assess the role of PYY on emesis induced by DON, fasted mink were first administered with the Y2R antagonist JNJ-31020028 (15 mg/kg bw) (Cippitelli *et al.*, 2011) or vehicle by sc injection at 8:45 h and immediately provided 50 g of feed. After 15 min, DON (0.25 mg/kg bw), $PYY_{3–36}$ (0.01 mg/kg bw, positive control), or PBS (negative control) was administered by ip injection. Mink were then returned to their cages and monitored for emesis over a 6-h period.

To determine if CCK contributed to DON-induced emesis, fasted mink were treated with the CCKR1 antagonist devazepide (0.1 mg/kg bw) (Richards *et al.*, 1996) or vehicle by sc injection at 8:30 h and provided 50 g of food. After 30 min, DON (0.25 mg/kg bw), CCK (0.0125 and 0.025 mg/kg bw), or PBS (negative control) was administered by ip injection and emesis monitored.

To ascertain the role of $5\text{-}HT_3$ in DON-induced emesis, fasted mink were administered with the $5\text{-}HT_3$ receptor antagonists granisetron (2 mg/kg bw) (Nakayama *et al.*, 2005) or PBS by ip injection at 8:30 h and provided 50 g of feed immediately after. After 30 min, DON (0.25 mg/kg bw), cisplatin (7.5 mg/kg bw, positive control), or PBS (negative control) was administered by ip injection, and mink were monitored for emesis. Cisplatin-treated mink were euthanized after the experiment because of the nephrotoxic effects of this drug.

To evaluate the effect of $PYY_{3–36}$ on $5\text{-}HT_3$-mediated emesis, mink were first administered with granisetron (2.5 mg/kg bw) or PBS by ip exposure at 8:30 h and provided 50 g of feed immediately thereafter. After 30 min, mink were administered with $PYY_{3–36}$ (0.01 mg/kg bw) or PBS via ip injection and mink were then monitored for emesis.

DON quantitation. Plasma was diluted 1:6 (vol/vol) in PBS and then centrifuged at 15,000 × g for 10 min. DON content in the supernatant fraction was determined using a Veratox High Sensitivity ELISA (Neogen, Lansing, MI) was performed as previously described (Pestka and Amuzie, 2008). Following addition of stop reagent (100 µl), plates were read on ELISA reader (Molecular Devices, Menlo Park, CA) at 690 nm and DON concentrations determined with Softmax software (Molecular Devices).

Plasma hormone measurement. Plasma hormones PYY and CCK were analyzed using enzyme immunoassay kits for PYY (PYY [3-36]; mouse, rat, porcine, and canine specific) and CCK (CCK [26-33], nonsulfated; human, rat, and mouse specific) (Phoenix Pharmaceuticals, Burlingame, CA). Plasma 5-HT was measured using a Serotonin EIA kit (Enzo Life Sciences, Plymouth Meeting, PA).

Data analysis. Data were plotted and statistically analyzed using SigmaPlot 11 for Windows (Jandel Scientific; San Rafael, CA). Means were considered significantly different at $p < 0.05$. A two-way ANOVA using Bonferroni *t*-test was used to assess significant differences in cumulative emetic events, kinetics of DON, PYY, and 5-HT concentrations in plasma. A one-way ANOVA using Tukey's test or *t*-test was used to analyze significant differences between treatments and the respective controls. If normality test failed, Kruskal-Wallis ANOVA on Ranks was used in conjunction with Student-Newman-Keuls test. The Spearman rank-order correlation coefficient was used for correlation between hormone levels and emetic events.

RESULTS

Mink Emetic Response Corresponds to DON Plasma Concentrations

Robust emesis was observed in mink exposed to 0.1 and 0.25 mg/kg bw DON (Fig. 1B) At the 0.1 mg/kg bw dose, most emesis occurred from 15 to 60 min. At the 0.25 mg/kg bw dose, 40, 41, 15, and 4% of emetic events were observed during 0- to 15-, 15- to 30-, 30- to 60-, and 60- to 120-min periods, respectively. Plasma DON concentrations in the 0.1 mg/kg group were highest after 15 min, whereas toxin concentrations peaked in the 0.25 mg/kg bw group at 30 min (Fig. 1C). The rate of emesis was greatest between 15 and 30 min, which coincided with maximal DON plasma concentrations for both doses.

$PYY_{3–36}$ Mediates DON-Induced Emesis

Exposure to DON at 0.25 mg/kg bw resulted in $PYY_{3–6}$ elevation at 30 and 60 min with a similar trend being evident at 0.1 mg/kg bw (Fig. 2A). Dose-dependent elevation of $PYY_{3–36}$ at 60 min correlated with cumulative emetic events (Fig. 2B). To further investigate the role of PYY in DON-induced emesis, mink were administered with either JNJ-31020028 or vehicle 15 min prior to exposure to DON (0.25 mg/kg bw), PYY, or

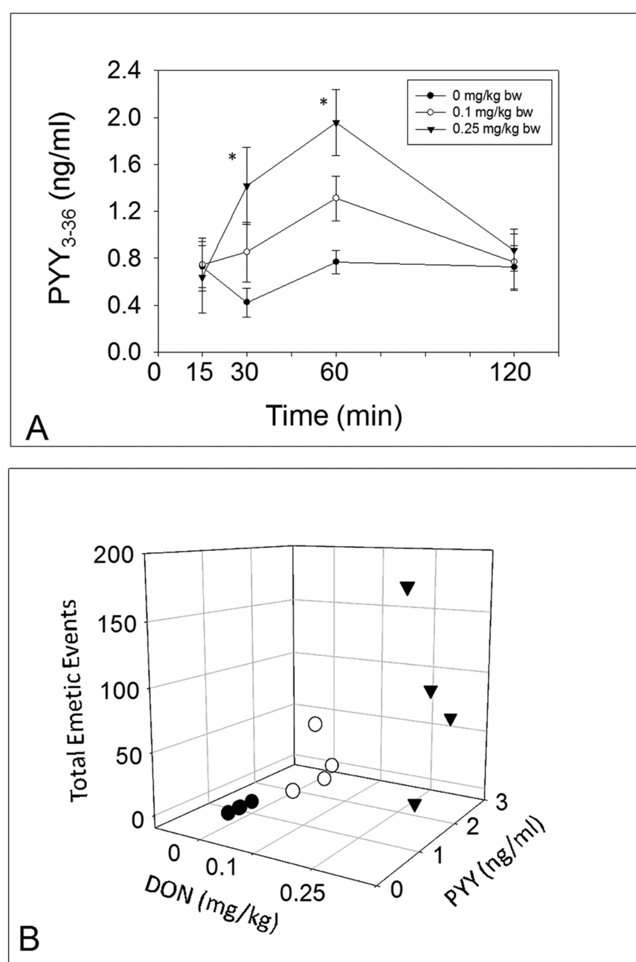

FIG. 2. Emesis induction corresponds to elevation in plasma $PYY_{3–36}$. (A) Kinetics of DON-induced plasma $PYY_{3–36}$ concentration. Data are the mean ± SEM ($n = 4$ per group). A two-way ANOVA using Bonferroni *t*-test was used to assess significant differences in kinetics of $PYY_{3–36}$ concentration in plasma. Asterisk indicates statistically significant differences in $PYY_{3–36}$ concentration compared with the control at $p < 0.05$. (B) Relationship between total emetic events and $PYY_{3–36}$ levels at 60 min in DON-treated mink. Cumulative emetic events significantly correlated with $PYY_{3–36}$ (Spearman rank-order correlation coefficient = 0.651, $p < 0.05$).

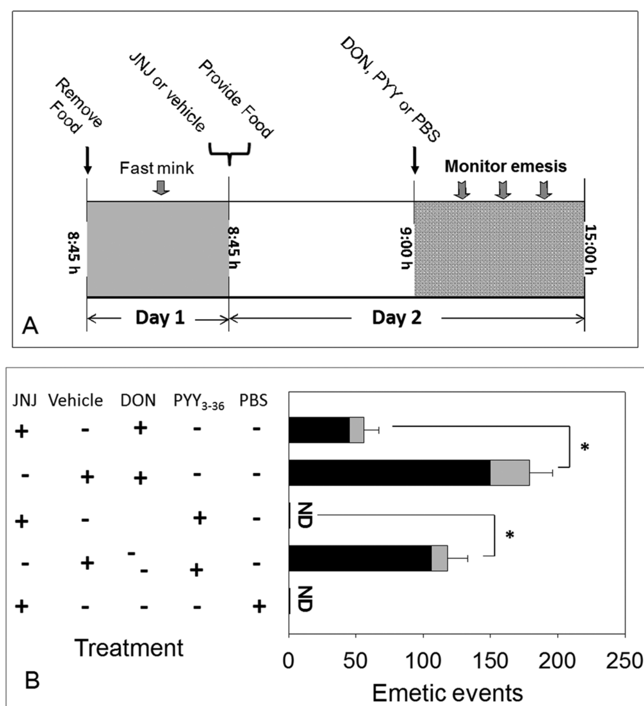

FIG. 3. PYY_{3-36} mediates DON-induced emesis. Experimental design for Y2 receptor antagonist in DON-induced emesis; (B) suppression of DON- and PYY-induced emesis by Y2 receptor antagonists. Emetic events include vomiting (gray) and retching (black) episodes. ND = not detected. Data represent mean ± SEM ($n = 4$ per group). A one-way ANOVA using Tukey's test or *t*-test was used to analyze significant differences between treatments and the respective controls. *$p < 0.05$ indicates statistically significant differences in emetic events between Y2 receptor antagonist treatment groups and only DON or PYY treatment group. These results and frequency, latency, and duration times are presented in tabular form in Supplementary data.

PBS (Fig. 3A). Mink treated with vehicle and then DON had 179 ± 17 total emetic events. Emetic events in mink administered with JNJ-31020028 prior to DON exposure were reduced by nearly 70%. JNJ-31020028 pretreatment completely suppressed PYY-induced emesis, confirming the efficacy of this drug. Animals pretreated with the drug and then vehicle did not exhibit emetic events. These results suggest that DON-induced emesis was mediated, in part, by PYY.

CCK Does Not Contribute to DON-Induced Emesis

Acute ip exposure to DON at 0.1 and 0.25 mg/kg bw had no effect on plasma CCK concentrations over the course of 120 min (data not shown). IP administration with CCK at doses of 0.0125 mg/kg bw did not induce emesis, whereas 0.025 mg/kg bw induced vomiting in one of three mink tested. CCK-induced emesis could be inhibited by pretreatment with the CCK1R antagonist devazepide; however, this drug had no effect on DON-induced emesis (Supplementary data). These observations indicate that CCK capacity to induce emesis was modest at best, and this hormone was unlikely to be a factor in emesis induction by this toxin.

5-HT Mediates DON-Induced Emesis

DON administered at 0.25 mg/kg bw induced an increase in plasma 5-HT concentrations at 60 min with a similar trend evident at 0.1 mg/kg bw DON (Fig. 4A). Cumulative emetic events at 60 min correlated with plasma 5-HT (Fig. 4B). The 5-HT_3 receptor antagonist granisetron was used to verify the role of 5-HT_3 receptor in DON-induced emesis (Fig. 5A). Pretreatment with PBS prior to DON dosing resulted in animals having 157 ± 22 total emetic events (Fig. 5B). However, administration of granisetron 30 min prior to DON exposure completely abolished this emetic response. Animals treated with PBS prior to cisplatin exhibited 79 ± 21 total emetic events, whereas emesis was not observed in animals treated with granisetron followed by cisplatin. These data suggest that both 5-HT and 5-HT_3 receptors are involved in DON-induced emesis.

5-HT Contributes to PYY-Induced Emesis

A final study was conducted to determine the relationship between PYY and 5-HT pathways of emesis (Fig. 6A). Animals pretreated with PBS prior to treatment with PYY exhibited 88 ± 14 total emetic events, whereas animals treated with granisetron prior to PYY had 37 ± 16 total emetic events (Fig. 6B). No significant differences in latency or duration of PYY-induced emesis were evident between animals pretreated with granisetron and animals pretreated with PBS. These data suggest that PYY's effects might be mediated, in part, by 5-HT.

DISCUSSION

Given the frequent presence of DON in grain-based foods and animal feeds, it is important to understand the basic mechanisms by which it causes gastrointestinal illness in humans and animals. The findings presented herein suggested that DON elevated plasma concentrations of 5-HT and PYY_{3-36}. Additionally, DON-induced emesis was inhibited by the 5-HT_3 receptor antagonist granisetron and the Y2R antagonist JNJ-31020028, further confirming roles for these hormones in DON-induced emesis. Thus, both hormones might cocontribute to DON-induced emesis in the mink (Fig. 7).

The data presented herein were consistent with a prior study demonstrating that 5-HT_3 receptors mediate DON-induced emesis in pigs (Prelusky and Trenholm, 1993). It is likely that DON induces exocytosis to EC cells, resulting in the release of 5-HT into the GI tract and subsequent stimulation of the 5-HT_3 receptors located on the vagal afferents. These stimulated vagal afferents would then activate the CPG via signaling through the NTS and AP, thereby evoking vomiting. In support of this contention, DON exposure delays gastric emptying (a surrogate for emesis) in rodents via a pathway involving 5-HT_3 receptors (Fioramonti *et al.*, 1993). Further consistent with our finding, Andrews *et al.* (1990) demonstrated that the trichothecene diacetoxyscirpinol (anguidine) caused emesis in ferrets that

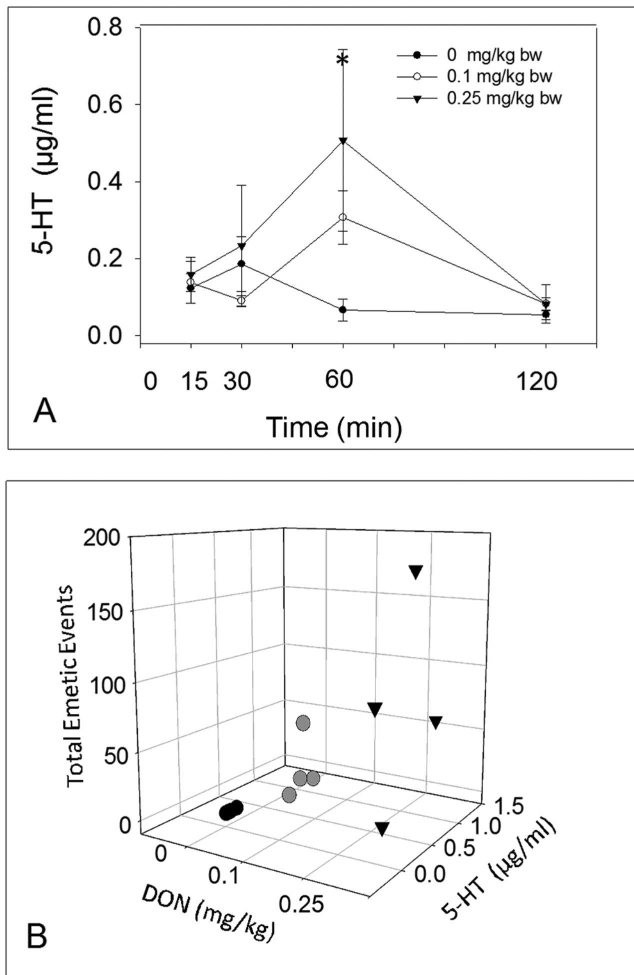

FIG. 4. Emesis induction corresponds to elevations in plasma 5-HT. (A) Kinetics of DON-induced plasma 5-HT. Data are the mean ± SEM ($n = 4$ per group). A two-way ANOVA using Bonferroni t-test was used to assess significant differences in kinetics of 5-HT concentration in plasma. Asterisk indicates statistically significant differences in 5-HT concentration compared with the control at $p < 0.05$. (B) Relationship between total emetic events and plasma 5-HT at 60 min. Cumulative emetic events significantly correlated with plasma 5-HT (Spearman rank-order correlation coefficient = 0.623, $p < 0.05$).

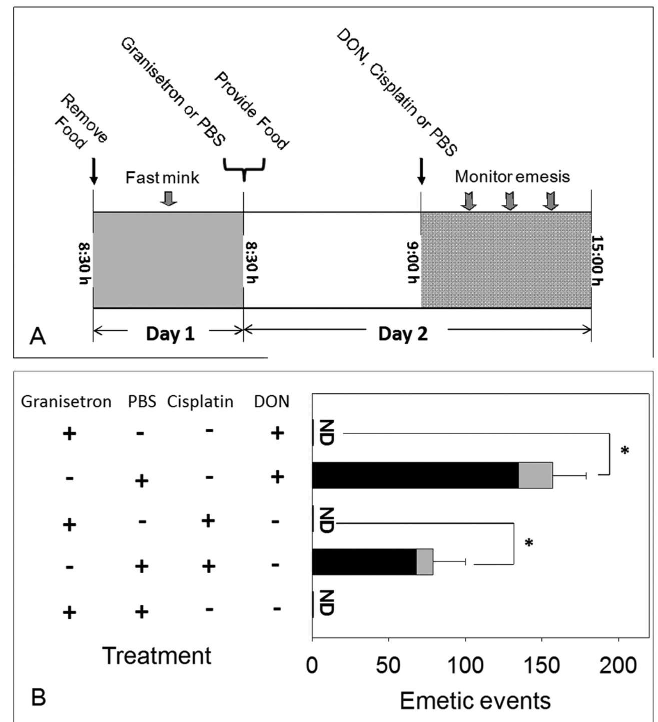

FIG. 5. 5-HT mediates DON-induced emesis. (A) Experimental design for 5-HT_3 receptor antagonist in DON- and cisplatin-induced emesis; (B) suppression of emesis by 5-HT_3 receptor antagonist, granisetron. Emetic events include vomiting (gray) and retching (black) episodes. ND = not detected. Data represent mean ± SEM ($n = 4$ per group). A one-way ANOVA using Tukey's test or t-test was used to analyze significant differences between treatments and the respective controls. Asterisk indicates statistically significant differences in emetic events between 5-HT_3 receptor antagonist treatment groups and only DON or cisplatin treatment group at $p < 0.05$. These results and frequency, latency, and duration times are presented in tabular form in Supplementary data.

could be attenuated by abdominal vagotomy or pretreatment with a 5-HT_3 receptor antagonist.

Though less studied for its emetogenic properties, PYY is one of the most potent peptide inducers of vomiting known (Harding and McDonald, 1989). Here, DON administration rapidly increased plasma concentrations of PYY_{3-36}, and furthermore, ip administration of PYY_{3-36} evoked emesis in mink, indicating that this peptide might indeed be a factor in the toxin's emetic effects. PYY_{3-36} could act by binding to Y2R located either on the vagal afferents or within the AP (Dumont *et al.*, 2007; Koda *et al.*, 2005), ultimately activating signaling within the CPG. Studies in the dog found that selective ablation of the AP could completely block PYY-induced emesis (Harding and McDonald, 1989). Because JNJ-31020028 penetrates the blood-brain barrier (Shoblock *et al.*, 2010; Swanson *et al.*, 2011), it has the capacity to block Y2Rs both centrally and peripherally, making it impossible to discriminate central versus peripheral actions of PYY at this time.

Given DON's capacity to robustly induce CCK release in mice (Flannery *et al.*, 2012), the potential of this gut satiety peptide to affect vomiting was also considered. Plasma CCK was not affected in mink as was previously observed in the mouse. Additionally, the ability of ip CCK administration to induce vomiting was quite modest in the mink as was previously observed in monkeys (Perera *et al.*, 1993) and ferrets (Percie du Sert *et al.*, 2012). Relatedly, Billig *et al.* (2001) found that CCK induced plasma vasopressin (arginine vasopressin), a physiological correlate of nausea in emetic species but not vomiting in the ferret. Although it is feasible that the CCK could still exert emetogenic effects without detectable changes in plasma concentrations, we were unable to reduce DON-induced emesis with the CCKR1 antagonist devazapide. Accordingly, these data do not support the possibility that CCK mediates DON-induced vomiting.

Although emesis corresponded with the kinetics of DON distribution and clearance in plasma, it was notable that an

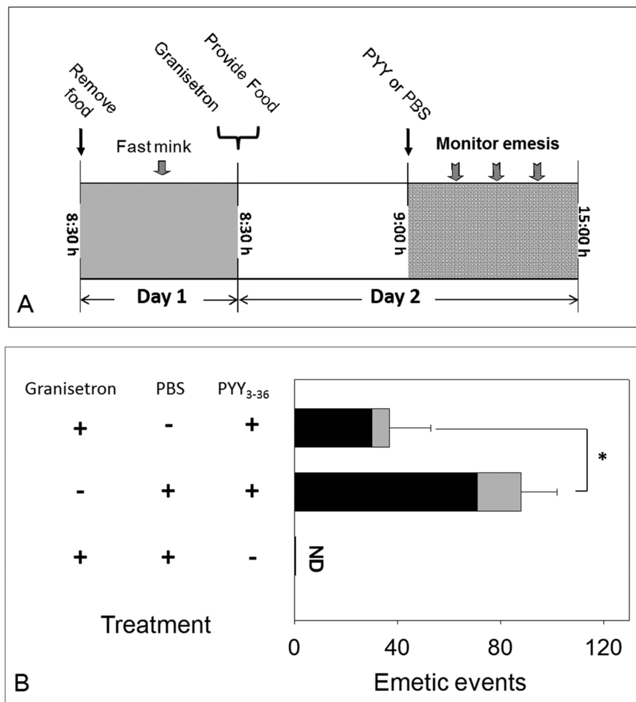

FIG. 6. PYY-induced emesis is mediated through 5-HT. (A) Experimental design; (B) suppression of PYY-induced emesis by 5-HT_3 receptor antagonist granisetron. Emetic events include vomiting (gray) and retching (black) episodes. ND = not detected. Data represent mean ± SEM (n = 4 per group). A one-way ANOVA using Tukey's test or t-test was used to analyze significant differences between treatments and the respective controls. Asterisk indicates statistically significant differences in emetic events between 5-HT_3 receptor antagonist treatment group and only PYY treatment group at $p < 0.05$. These results and frequency, latency, and duration times are presented in tabular form in Supplementary data.

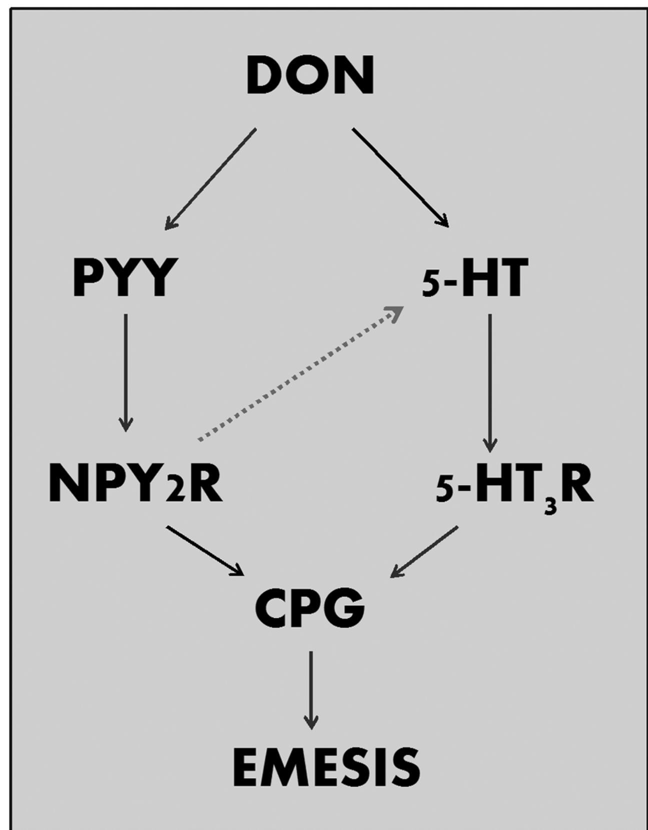

FIG. 7. Putative mechanisms for DON-induced emesis. The results presented here suggest that DON could act by inducing PYY release (e.g., L cells) and 5-HT release (e.g., EC cells). These might activate neuropeptide Y2 receptor (NPY2R) and 5-HT_3 receptors (5-HT_3R), respectively, in the peripheral and central nervous systems, ultimately inducing emesis via the CPG. The potential exists for significant crosstalk (dotted line)ss whereby PYY induces 5-HT release at peripheral and central sites.

elevation of 5-HT plasma concentration followed rather than preceded the onset and peak of emetic activity. Plasma 5-HT elevation was only detectable at 60 min after DON administration at the highest dose, which differs with the prior findings showing no detectable changes in plasma 5-HT in pigs in response to DON (Prelusky, 1994). Although species differences, sampling times, and improvements in assay sensitivity could explain these differing results, other factors might be involved. Most (> 90%) of the total 5-HT is located in the GI tract within EC cells and enteric neurons (Feldberg and Toh, 1953; Kim and Camilleri, 2000; Resnick and Gray, 1961), with the remainder being found in the brain (Endo *et al.*, 2000). Once 5-HT is released from EC cells, most 5-HT will be metabolized into 5-HIAA within the gut wall or liver; however, increased 5-HIAA in plasma or urine does not correlate with circulating 5-HT concentrations (Endo *et al.*, 2000). Additionally, circulating 5-HT can be effectively up taken by platelets, leading to no increase in plasma concentrations (Thomas and Vane, 1967). Interestingly, in a chemotherapy-induced emesis study, circulating 5-HT was not associated with emesis induction by cisplatin, even though this adverse effect that could be countered with 5-HT_3 receptor antagonists (Castejon *et al.*, 1999).

It is particularly intriguing that plasma PYY was elevated (30 min) prior to 5-HT (60 min) and thus somewhat more in synchrony with emesis. Although it is possible that 5-HT release occurs earlier but is masked by rapid metabolism to 5-HIAA, this finding along with our observation that granisetron attenuated PYY-induced emesis suggests that this satiety peptide might indeed be capable of inducing 5-HT-mediated events. Two analogous prior studies support this contention. First, it has been established in the fistulated dog that iv administration of either PYY or 5-HT can reduce intestinal transit and, furthermore, PYY-induced suppression of intestinal transit can be blocked by the 5-HT_3 receptor antagonist ordansetron (Lin *et al.*, 2004). Second, studies using guinea pig colonic mucosal sections revealed that exogenously applied PYY induced sustained release of 5-HT that could be blocked with NPY receptor antagonists (Kojima *et al.*, 2012). It was also shown that selective tachykinin NK2 receptor agonism evoke PYY and 5-HT release in the mucosal sections and that the latter can be

suppressed with NPY receptor antagonists. Collectively, these findings suggest that PYY-containing L cells could control, in part, the release of 5-HT from intestinal EC cells (Fig. 7). Interestingly, Perry *et al.* (1994) have previously reported that plasma PYY and 5-HT are elevated in cisplatin-treated dogs. However, granisetron pretreatment impaired elevation of PYY but not 5-HT, suggesting different mechanisms are in play for this drug and model.

Although much of the present discussion focuses on gut endocrine cells as DON targets, it cannot be excluded that the toxin directly targets the AP or central nervous system to modulate emetic neurocircuitry by stimulating PYY and 5-HT release in the brain. Consistent with a possible direct action of DON on the central nervous system, we previously observed that the toxin is detectable in the brain of mice within 5 min of oral exposure (Pestka *et al.*, 2008). Furthermore, po administration of DON can induce c-Fos expression in the circumventricular organs of the brain (Girardet *et al.*, 2011a,b). Recent immunohistochemical studies have shown the colocalization of PYY and 5-HT within neurons and that PYY-producing cells are present in the hindbrain (Gelegen *et al.*, 2012) with 5-HT fibers in close apposition suggesting possible synaptic contacts. Therefore, the possibility that DON has direct effects on PYY and 5-HT in brain merits further investigation.

To summarize, the results provided herein suggest that PYY and 5-HT are prominent mediators of DON-induced emesis. Future studies should focus on discerning how DON and other trichothecenes act on L-cells and EC-cells within the gut and neurons within the brain. It will be of additional interest to understand how and where PYY affects serotonergic pathways. Strategies for such approaches could include (1) measurement of residual intestinal mucosal 5-HT and PYY following DON treatment, (2) determination of whether DON can cause direct release of 5-HT and PYY from intestine *in vitro*, (3) use of peripheral 5-HT hydroxylase inhibitors, and (4) ascertaining the effect of selective nerve lesions on DON-induced emesis. Ultimately, such studies will improve our understanding of how DON and other trichothecenes cause human food poisoning.

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