Anorexia Induction by the Trichothecene Deoxynivalenol (Vomitoxin) Is Mediated by the Release of the Gut Satiety Hormone Peptide YY

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Consumption of deoxynivalenol (DON), a trichothecene mycotoxin known to commonly contaminate grain-based foods, suppresses growth of experimental animals, thus raising concerns over its potential to adversely affect young children. Although this growth impairment is believed to result from anorexia, the initiating mechanisms for appetite suppression remain unknown. Here, we tested the hypothesis that DON induces the release of satiety hormones and that this response corresponds to the toxin’s anorectic action. Acute ip exposure to DON had no effect on plasma glucagon-like peptide-1, leptin, amylin, pancreatic polypeptide, gastric inhibitory peptide, or ghrelin; however, the toxin was found to robustly elevate peptide YY (PYY) and cholecystokinin (CCK). Specifically, ip exposure to DON at 1 and 5 mg/kg bw induced PYY by up to 2.5-fold and CCK by up to 4.1-fold. These responses peaked within 15–120 min and lasted up to 120 min (CCK) and 240 min (PYY), corresponding with depressed rates of food intake. Direct administration of exogenous PYY or CCK similarly caused reduced food intake. Food intake experiments using the NPY2 receptor antagonist BIE0246 and the CCK1A receptor antagonist devazepide, individually, suggested that PYY mediated DON-induced anorexia but CCK did not. Orolingual exposure to DON induced plasma PYY and CCK elevation and anorexia comparable with that observed for ip exposure. Taken together, these findings suggest that PYY might be one critical mediator of DON-induced anorexia and, ultimately, growth suppression.

Key Words: deoxynivalenol; anorexia; peptide YY; cholecystokinin; mouse.

Fungal infection of crops in the field and during storage often leads to the production of mycotoxins, harmful secondary metabolites that can cause adverse health consequences in humans and animals. Deoxynivalenol (DON; vomitoxin), a trichothecene mycotoxin produced by Fusarium sp., is of particular public health concern because it contaminates grains with high frequency, is resistant to cooking processes, and has been associated with both animal and human illnesses (Pestka, 2010). The mouse and the pig have been most widely used to study DON toxicity. Following oral DON exposure in these species, the toxin is absorbed extremely rapidly and distributed throughout body including the gut (Azcona-Olivera et al., 1995; Pestka and Amuzie, 2008; Prelusky et al., 1988). In mice, which are incapable of vomiting, acute DON exposure is associated with anorexia and proinflammatory cytokine induction, whereas subchronic and chronic DON exposure causes growth suppression and weight loss (Canady et al., 2001). In pigs, which have an emetic response, DON elicits the aforementioned effects and vomiting (Forsyth et al., 1977; Pestka et al., 1987). Interestingly, the Joint Expert Committee on Food Additives of the WHO and FAO established a human tolerable daily intake (TDI) of 1 μg/kg bw/day for DON based on growth suppression in mice (Canady et al., 2001). This TDI now serves as the basis for current regulatory tolerances for DON in grain-based foods.

Although experimental animal studies strongly suggest that DON-induced growth suppression results from reduced food intake (Arnold et al., 1986; Goyarts et al., 2005; Hughes et al., 1999), the mechanisms by which the toxin causes anorexia still remain poorly understood. The regulation of food intake is multifaceted and reflects both central and peripheral neuroendocrine control mechanisms. Centrally, the balance of orexigenic (e.g., neuropeptide Y [NPY], agouti-related protein [AGRP]) and anorexigenic signaling molecules (e.g., pro-opiomelanocortin [POMC], cocaine- and amphetamine-regulated transcript [CART]) produced within hypothalamic neurons are critical for food intake control (Schwartz, 2006). Based on a recent elegant study, it has now been established that DON exposure upregulates hypothalamic mRNA expression of signaling molecules CART, POMC, and one of the receptors through which they have their action, the melanocortin 4 receptor (Girardet et al., 2011b). However, it remains to be established how DON modulates expression of these anorexigenic signaling molecules.

One possible mechanism for the initiation of anorexia induction by DON is through upregulation of proinflammatory genes. DON can stimulate expression of cyclooxygenase-2, microsomal...
prostaglandin synthase-1, tumor necrosis factor-alpha, interleukin-1, and interleukin-6 in many tissues in mice including the brain (Girardet et al., 2011a; Pestka and Amuzie, 2008), and our laboratory has established that the underlying mechanism for this upregulation is ribotoxic stress in mononuclear phagocytes (Pestka, 2010). These proinflammatory gene products are well known to mediate anorexia through classic sickness behavior mechanisms (Kelley et al., 2003) and potentially can impair insulin-like growth factor expression (Amuzie and Pestka, 2010). However, the dose thresholds and the kinetics for proinflammatory gene upregulation in vivo (2h) (Zhou et al., 1997) are not congruent with the rapid initiation of anorexia (Flannery et al., 2011). Moreover, food intake and growth are unaffected by dietary DON in mice deficient in these genes, suggesting that DON might additionally act through other mechanisms different from those associated with endotoxin exposure or infection (Girardet et al., 2011a; Jia and Pestka, 2005; Pestka and Zhou, 2000, 2002).

An alternative unexplored possibility is that satiety-regulating hormones produced by enteroneuroendocrine cells in the gastrointestinal system might serve as early mediators of DON-induced anorexia. Secretion of satiety hormones represents an important mechanism for peripheral control of the balance of hypothalamic orexigenic and anorexigenic signaling. Although serotonin, which can be produced at the gut level, is one obvious candidate gut hormone, changes in plasma levels of this neurotransmitter were not detectable in DON-exposed pigs (Prelusky, 1994). Other possible gut hormones that affect appetite include glucagon-like peptide-1 (GLP-1), leptin, amylin, pancreatic polypeptide (PP), gastric inhibitory peptide (GIP), ghrelin, peptide YY (PYY) and cholecystokinin (CCK). The latter two peptides are particularly attractive candidates for mediating DON’s anorectic actions because the rapidity by which they decrease food intake (Gibbs et al., 1976; Moran et al., 2005) mimics that observed following acute DON exposure (Flannery et al., 2011).

PYY is a 36-amino acid protein released from the L cells of the colon and ileum that signals reductions in food intake by increasing expression of anorexigenic peptides and decreasing orexigenic signaling peptides within the hypothalamus (Challis et al., 2003). CCK is a peptide hormone that is secreted by the small intestine that acts on vagal afferent neurons to increase expression of anorexigenic peptides including CART though recent research suggests that CCK can act directly within the brain as well (Brown et al., 2011; de Lartigue et al., 2007). Thus, DON-induced release of PYY and CCK from the gastrointestinal tract could promote signaling changes observed within the hypothalamus (Girardet et al., 2011b).

The purpose of this study was to test the hypothesis that DON induces the release of gut satiety hormones in the mouse and that this response corresponds to the toxin’s anorectic action. The results demonstrate that acute exposure to DON at levels of 1–5 mg/kg bw caused release of PYY and CCK and that kinetics of these responses corresponded to onset and duration of feed refusal. Although direct administration of both hormones similarly evoked transient anorexia, experiments using receptor inhibitors suggest that PYY might play a more dominant role in DON-induced anorexia. Accordingly, aberrant release of gut satiety hormones might be one critical underlying mechanism for DON-induced anorexia and ultimately growth suppression.

MATERIALS AND METHODS

Animals. Naïve female B6C3F1 mice (9–11 weeks) were purchased from Charles River Breeding (Portage, MI) and allowed to acclimate at least 1 week prior to beginning experiments. During acclimation, mice were adapted to high fat diet (45% kcal from fat; Research Diets, Inc., New Brunswick, NJ) and conditioned by frequent handling and sham injections to mimic experimental protocols. Mice were maintained at constant temperature and humidity (21°C–24°C and 40–55%, respectively) under 12-h light/dark cycles with free access to food and water. All experiments performed and were approved by the Institute of Animal Care and Use Committee at Michigan State University and in accordance with the National Institutes of Health guidelines for animal use.

DON administration. DON was obtained from Dr Tony Durst (University of Ottawa, Canada) and purity verified using 1H NMR and carbon and hydrogen elemental analysis (Galbraith Labs, Knoxville, TN). The ip route of DON exposure was used for most experiments to ensure accurate and reproducible delivery of the toxin and to minimize handling stress that might artifactually raise plasma hormone concentrations. DON was dissolved at appropriate concentrations in 100 μl PBS and administered via ip injection using sterile 31G, ½ inch syringes. For a comparative study using orolingual exposure, DON was dissolved in 10 μl PBS and administered to the mouth and tongue using a 20 μl sterile filter pipette tip.

DON-induced gut satiety hormone release. For preliminary studies, groups of mice were treated with 2.5 and 10 mg/kg bw DON for 15 and 60 min. For the initial study on PYY and CCK, groups of mice were given an ip injection of 0, 0.05, 0.25, 1, or 5 mg/kg bw DON and sacrificed at 15 and 240 min after DON exposure. For kinetic studies, groups of mice were given an ip injection of 0, 1, or 5 mg/kg bw and sacrificed at 15, 60, 120, and 180 min for 1 mg/kg bw dose and at 30, 120, 240, and 360 min for 5 mg/kg bw DON exposure. The control group was given PBS and sacrificed at 0, 15, 30, and 120 min to confirm that PYY and CCK levels remain stable. These time points were chosen based on data from Flannery et al. (2011) showing that feed refusal was attenuated by 180 min at 1 mg/kg bw and by 360 min at 5 mg/kg bw DON. For ip versus orolingual studies, mice were given 2.5 mg/kg bw DON or PBS and sacrificed at 30 min.

For all experiments, mice were euthanized by ip injection with 56 mg/ml bw sodium pentobarbital (100 μl) or 100 mg/ml ketamine (40 μl). Blood was removed from the inferior vena cava using sterile BD syringes with 25 μl of 1% (vol/vol) EDTA (pH 7.5) and placed into EDTA-coated tubes. Plasma was separated by centrifugation at 3500 × g for 10 min at 4°C and then frozen in aliquots at −80°C until hormone analyses.

Hormone analyses. Plasma PYY (1–36), GLP-1, leptin, amylin, PP, GIP, and ghrelin were measured using a Milliplex Maps Kit according to manufacturer’s instructions (Mouse Gut Hormone Panel, no. MGT-78K; Millipore, St Louis, MO). Plates were read using a Bio-Rad Bioplex 100 system and data analyzed from a standard curve using Bioplex Manager 5.0 (Bio-Rad Life Science, Hercules, CA). CCK was analyzed using a Cholecystokinin Octapeptide (26–33, nonsulfonated) Extra Sensitive EIA Kit (no. EKE-069-04; Phoenix Pharmaceuticals, Inc., Burlingame, CA). Plate absorbance was read at 450 nm using an ELISA plate reader (Molecular Devices, Menlo Park, CA), and data were analyzed using Softmax software.

DON- and hormone-induced anorexia. DON-induced anorexia was assessed in conditioned mice by measuring food intake at 1, 2, 4, 6, and 16 h.
after ip exposure and 2h after orolingual exposure under red light conditions according to our previously described protocol (Flannery et al., 2011). For hormone-induced anorexia, PYY (3–36) was purchased from Tocris Biosciences (Ellisville, MO) and CCK (23–33, sulfonated) from Sigma-Aldrich (St Louis, MO) and dissolved in PBS for use. Food intake was assessed at 1, 2, and 6h after ip injection with 100 μg/kg bw PYY or 15, 30, 60, and 120min after ip injection with 8 μg/kg bw CCK. Measurement times were based on reports that anorectic effects of PYY last longer than CCK (Adrian et al., 1985; Liddle et al., 1986). Doses were chosen based on previously reports that these consistently induce robust anorexia (Parkinson et al., 2008; Weatherford et al., 1992).

Receptor antagonist effects on DON-induced anorexia. The effect of receptor antagonists on DON-induced anorexia was assessed as described above with the exception that fresh food was given for the first two measurements to minimize the time the animals were without food. Also, to reduce animal usage, a cohort of mice was used for experiments with the respective hormone and inhibitor and then the same cohort rested at least 5 days, randomized, and used in inhibitor plus DON experiments.

To confirm that the NPY2 receptor antagonist BIE0246 (Tocris Bioscience, Minneapolis, MN) attenuated PYY-induced anorexia, 100 μl of 7% dimethyl sulfoxide (DMSO) in PBS (control) or 1.67 mg/kg bw BIE0246 in 7% DMSO was given via ip injection 30min before the ip administration of 50 μl PBS or 100 μg/kg bw PYY. Food intake was measured at 30, 60, and 120min. To establish the role of PYY in DON-induced anorexia, mice were given an ip injection of 100 μl of 7% DMSO in PBS (control) or 1.67 mg/kg bw BIE0246 in 7% DMSO 30min before the administration of 50 μl PBS or 1 mg/kg bw DON in PBS. Food intake was subsequently measured at 30, 60, and 120min. This PYY experiment was performed twice to confirm findings, and data were combined.

To verify that the CCK1A receptor antagonist devazepide (DEV; Tocris Bioscience) attenuated CCK-induced anorexia, 50 μl of either 3% DMSO in PBS (control) or 0.6 mg/kg bw DEV in 3% DMSO was given via ip injection. Thirty minutes later, mice were injected ip with 8 μg/kg bw CCK in PBS. Food intake was measured at 15, 30, 60, and 120min. To ascertain a potential role of CCK in DON-induced anorexia, mice were given an ip injection of 50 μl of either 3% DMSO in PBS (control) or 0.6 mg/kg bw DEV in 3% DMSO. Thirty minutes later, mice were injected ip with 50 μl PBS or 1 mg/kg bw DON in PBS. Food intake was then measured at 30, 60, and 120min.

Statistics. Data were analyzed using SigmaPlot 11.0 (Systat Software, San Jose, CA). Student’s t-test was used to establish statistical significance between two independent groups (p ≤ 0.05). To determine statistical significance for multiple groups, one-way ANOVA with Holm-Sidak multiple comparisons test was used (p ≤ 0.05). If normality failed, a Kruskal-Wallis one-way ANOVA was used (p ≤ 0.05). For multiple groups, one-way ANOV A with Holm-Sidak multiple comparisons test was employed to establish statistical significance among groups and time points (p ≤ 0.05).

Samples receiving two treatments (receptor antagonist plus DON) were analyzed for additivity, potentiation, or antagonism by randomly combining single treatment replicates to calculate a predicted mean additive response with variance as described previously (Zhou et al., 2000). This calculated value was compared with actual cotreated samples using a Mann-Whitney rank sum test.

RESULTS

DON Exposure Induces Elevations in PYY and CCK but Not Other Appetite-Regulating Hormones

Preliminary studies using a gut hormone panel revealed that acute ip exposure to DON at 2.5 and 10mg/kg bw had no effect on plasma GLP-1, leptin, amylin, PP, GIP, or ghrelin at 15 and 60min; however, these treatments markedly increased plasma PYY and CCK (data not shown). Based on these findings, the effects of DON doses of 0, 0.05, 0.25, 1.0, and 5mg/kg on plasma levels of PYY and CCK were assessed (Figs. 1 and 2). Exposure to 5mg/kg DON significantly increased plasma PYY relative to the vehicle control value at 15min, and this elevation was still evident after 240min (Fig. 1). At 15min, a similar trend toward elevated PYY was observed for 1mg/kg bw DON, compared with vehicle control (p = 0.053). Administration of 1 and 5mg/kg DON also significantly increased plasma CCK, respectively, compared with vehicle-treated controls at 15min but not 240min (Fig. 2).

Based on the observed induction of PYY and CCK by DON exposure at 1 and 5mg/kg bw, the kinetics of responses to these two doses were further investigated. At 1mg/kg bw, plasma PYY was approximately twice that of the zero time control from 15 to 180min after DON treatment (Fig. 3). Following exposure to 5mg/kg bw, plasma PYY remained significantly elevated (2.0- to 2.5-fold) through 240min but not at 360min.

FIG. 1. DON induces plasma PYY elevation. Mice were treated ip with various doses of DON and plasma was analyzed for PYY after 15 and 240min. Data (n = 5–6/gp) are mean ± SEM. * indicates a statistically significant difference relative to the control (p ≤ 0.05); # indicates p = 0.053.

FIG. 2. DON induces plasma CCK elevation. Mice were treated ip with various doses of DON and plasma was analyzed for CCK after 15 and 240min. Data (n = 5–6/gp) are mean ± SEM. * indicates a statistically significant difference relative to the control (p ≤ 0.05).
compared with the zero time control. Plasma CCK increased rapidly in response to both 1 and 5 mg/kg bw DON (Fig. 4). At 1 mg/kg bw DON, plasma CCK was 2.6- and 2.7-fold the zero time control at 15 and 60 min, respectively, and returned to zero time control levels by 120 min. At 5 mg/kg bw DON, plasma CCK increased nearly fourfold at 30 and 120 min but returned to zero time control levels by 240 min. Finally for both hormones, vehicle treatment had no effect at 15, 30, and 120 min, suggesting that their initial induction during the first 2 h was not related to handling or the injection procedure (Figs. 3 and 4).

**DON Exposure Rapidly Induces Anorexia**

To relate PYY and CCK responses to DON-induced anorexia, food consumption was monitored over intervals for 16 h after ip exposure to 1 and 5 mg/kg bw DON. Total food intake in mice exposed to 1 mg/kg bw DON was reduced by 58 and 42% compared with control mice at 1 and 2 h, respectively, but recovered by 4 h (Fig. 5). Food intake by mice exposed to 5 mg/kg bw at 1, 2, and 4 h was reduced by 86, 92, and 87%, respectively, relative to the control. By 6 h, mice began eating more relative to earlier measurements, but cumulative intake was still depressed by 51%. By 16 h, cumulative food intake returned to control levels.

Both Orolingual and ip DON Exposure Induce PYY, CCK, and Anorectic Responses

The effects of orolingual exposure to DON at 2.5 mg/kg bw on hormone release and anorexia induction were measured and compared with those caused by ip exposure. Treatment with DON by the orolingual route elevated plasma PYY and CCK by 2.1- and 2.5- fold, respectively, after 30 min and suppressed food intake by 67% (Table 1). These responses were comparable with effects observed following ip exposure to identical DON dose. Taken together, both orolingual and ip exposure similarly affected gut satiety hormone release and anorectic responses, and this might relate to DON’s capacity to be absorbed and distributed rapidly by either route of exposure.

Exogenous PYY and CCK Suppress Food Intake

Mice were treated with exogenous PYY and CCK to confirm that these hormones could indeed evoke satiety responses in the female B6C3F1 mouse. The doses chosen were consistent with those previously established that consistently evoke anorexia in mice. At 1, 2, and 6 h, PYY significantly reduced food intake by 42, 39, and 11%, respectively, relative to control levels (Fig. 6). CCK’s anorectic effects did not last as long as those of PYY.
Relative to the control, CCK significantly reduced food intake by 90% at 15 min and 46% at 30 min, with food intake no longer statistically different from control values by 60 min (Fig. 7).

NPY2 Receptor Antagonist BIIE0246 Interferes With PYY- and DON-Induced Anorexia

The effects blocking the NPY2 receptor in PYY- and DON-induced food refusal were evaluated. PYY decreased food intake by 54, 41, and 35% relative to the control at 30, 60, and 120 min, respectively (Fig. 8A). These effects were attenuated with BIIE0246, thus confirming the action of the inhibitor. When mice were treated with DON or BIIE0246 alone, food consumption over 60 min was reduced by 56 and 17%, respectively (Fig. 8B). Although the predicted reductions in food intake from DON and BIIE0246 cotreatment over this time period was 71%, the actual observed reduction was 33%. This strong trend \((p = 0.059)\) toward reversal of DON-induced anorexia in mice upon NPY2 receptor antagonism suggests that PYY might play an important role in this effect.

CCK1A Receptor Antagonist DEV Ablates CCK-Induced but Not DON-Induced Anorexia

Mice treated with CCK exhibited a significant reduction in food intake at 15 and 30 min with similar trend being evident at 60 min (Fig. 9A). CCK-induced food reduction was ablated prior to DEV administration. To ascertain a possible role for CCK in DON-induced anorexia, mice were pretreated with DEV before toxin exposure. Mice exposed to DON consumed 25% less over 30 min, whereas mice receiving DEV consumed 34% more food over the same period (Fig. 9B). DON-exposed mice that were pretreated with DEV exhibited a trend of increased food intake compared with mice exposed to DON alone. However, when the predicted combined effects of the antagonist and DON were compared with the actual response, no differences were evident. Therefore, the attenuation exhibited in DEV pretreated DON-exposed mice appeared to result from DEV blocking basal CCK from interacting with CCK1R.

**DISCUSSION**

DON’s capacity to impair food intake and suppress growth has long been of major concern for both the human and animal health fields; however, the underlying mechanisms for these clinical effects are still relatively poorly understood. Our findings here demonstrate, for the first time, that DON rapidly induces plasma levels of two major gut satiety hormones PYY and CCK in the mouse and that this induction occurs in
a manner remarkably consistent with anorexia induction. In addition, like DON, administration of exogenous PYY and CCK caused immediate food refusal. While NPY2 receptor antagonism attenuated DON-induced anorexia, CCK1 receptor antagonism did not, suggesting that PYY might play a more important role than CCK in DON-induced anorexia. These findings have public health significance because they suggest DON’s anorectic effects might, in part, be hormone-driven and linked to an aberrant postprandial satiety response.

Satiety is a complex process involving (1) release of hormones such as PYY and CCK from the gastrointestinal tract, (2) changes in the expression of orexigenic and anorexigenic signaling peptides within the arcuate nucleus of the hypothalamus, and (3) alterations in gut motility and hormone secretion. Because DON caused a release of satiety hormones, it might be speculated that the toxin caused mice to be satiated and, thus, prevented food intake. These findings are consistent with those of two research groups who have shown that DON exposure leads to the “fed pattern” of gut motility in rats (Fioramonti et al., 1993; Krantis and Durst, 2001). Thus, DON-exposed mice might refuse food in part because they feel full.

Upon consuming a meal, both PYY and CCK increase rapidly with circulating plasma levels elevated within 15 min for PYY in humans and 5 min for CCK. DON intake was then measured at various intervals. Data are mean ± SEM (n = 5–7/gp). For both graphs, different letters indicate a statistically significant difference between treatment groups for that time point (p ≤ 0.05). Plasma PYY in animals receiving DON and receptor antagonist were further compared with the predicted mean additive PYY response as described in methods (p = 0.059).

Comparison of plasma CCK in animals receiving DON and receptor antagonist to the predicted mean additive CCK response indicated that the difference was not significant (n.s.).
mice recovered from DON-induced anorexia, whereas plasma PYY concentrations remained elevated even after cessation of feed refusal. It is possible that elevated plasma PYY no longer impacted food intake because of the desensitization, saturation, and rate of cycling of NPY2 receptors or because other hormones modulated sensitivity of NPY2 receptors to PYY (Parker and Balasubramaniam, 2008).

While modest food consumption results in satiation, overconsumption could lead to excessive fullness, nausea, and even vomiting (in susceptible species). Interestingly, PYY is extremely potent in its ability to cause dogs to vomit, and its elevation in plasma has also been associated with cisplatin-induced emesis (Harding and McDonald, 1989; Perry et al., 2005; Gantz et al., 2007). Postprandially, lean mice given a 2.6% fat meal exhibited approximately a 20% increase over basal plasma levels of PYY after 120 min (le Roux et al., 2006). We observed here that unfed mice exposed to DON exhibited a remarkably greater (~twofold) increase in plasma PYY levels over control values at both 1 and 5 mg/kg bw. Thus, the PYY response in DON-exposed mice was far greater than that reported previously from meal consumption. It might be speculated that, in emesis-capable species, such a large increase in PYY (i.e., relative to meal-induced) might lead to vomiting. Further studies are, therefore, warranted on the role of PYY in DON-induced emesis in an appropriately sensitive species.

Here, the use of the antagonists of the NPY2 and CCK1 receptors revealed that PYY plays a more important role than CCK in DON-induced anorexia. Unlike CCK, PYY can penetrate the blood-brain barrier to affect signaling changes within the hypothalamus, and CCK acts through the vagus nerve to evoke changes in food intake (Moran et al., 1997). Consistent with this mechanism, Girardet and coworkers (2011b) showed that upon a cervical vagotomy, mice dosed with oral DON still exhibited changes in c-Fos expression within the brainstem. Although food intake was not measured in that particular experiment, these data suggest the vagus nerve was not necessary for C-Fos activation and may not be necessary for hypothalamic signaling or food intake changes caused by DON.

The observation that the NPY2 receptor antagonist could not completely inhibit DON-induced anorexia might relate to the distribution kinetics of BIIE0246 and the complexity of appetite regulation. Administration of BIIE0246 by the ip route in the mouse resulted in observed brain concentrations being 2% of those found in the plasma (Brothers et al., 2010). Therefore, only small amounts of BIIE0246 could have entered the brain of DON-exposed mice, leading to only a modest effect of BIIE0246 on DON-induced anorexia. Higher doses of BIIE0246 were not used in these experiments because we observed that these doses caused ataxia in preliminary experiments. It should be emphasized that food intake is so critical for survival that many redundant and complex pathways exist to regulate appetite. Furthermore, food intake behavior is affected by social factors, such as housing, or emotional factors, such as the stress of handling, that play a role in food intake changes (Schwartz et al., 2000). Thus, suppression of food intake inhibition as observed with BIIE0246 on DON-induced feed refusal would likely be of biological significance. Further experiments are needed to elucidate the role of the brain in the peripheral release of gut satiety hormones in the DON feed refusal model.

In conclusion, the heretofore unreported findings presented here highlight a potential new mechanism by which DON causes anorexia and, as a result, growth suppression. Secretion of PYY may serve as a defense mechanism for preventing continued ingestion of toxic DON by suppressing appetite and decreasing gut motility. Abrerrant release of PYY could have further potential to contribute to DON-induced emesis in animal species capable of a vomiting response. Thus, important future considerations will be to determine whether the release of PYY contributes to the reported vomiting caused by DON. In addition, gut satiety hormones are reportedly elevated in eating disorders related to conditions such as old age (Silver et al., 1988), chronic gastrointestinal illnesses (Chua et al., 2006; Khoo et al., 2010; Van Der Veek et al., 2006), cancer cachexia (Moschovi et al., 2008), and anorexia/bulimia (Germain et al., 2007; Lawson et al., 2011). It will, therefore, be of interest to discern if these disease states and other susceptibility factors might further heighten an individual’s sensitivity to DON or other trichotheccenes. Finally, the exact mechanisms for robust PYY and CCK responses to DON are not known. A plausible hypothesis is that these gut satiety hormones are released from enteroendocrine cells as an evolutionary response to toxic substances (Glendinning, 2007) to diminish and prevent further ingestion of the agent. Future work should focus on the role of specific receptors in mediating this response.

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