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Role of neurotransmitters 5-hydroxytryptamine and substance P in anorexia induction following oral exposure to the trichothecene T-2 toxin

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ABSTRACT

Trichothecene mycotoxins, a family of common contaminants on cereal grains, are known to negatively impact human and animal health with adverse effect on food consumption being of particular concern. T-2 toxin has been previously demonstrated to induce anorectic response in several animal species including mouse, rat, rabbit. Although the T-2 toxin-induced anorectic response has been associated with the release of gut satiety hormone, much less is known about the role of neurotransmitter in this response. To address this gap, we employed a nocturnal mouse food refusal model to test the hypothesis that neurotransmitters 5-hydroxytryptamine (5-HT) and substance P (SP) mediate anorexia induction by T-2 toxin. Elevations of plasma 5-HT and SP markedly corresponded to anorexia induction following oral exposure to T-2 toxin. Direct administration of exogenous 5-HT and SP induced anorectic responses similar to T-2 toxin. The 5-HT3 receptor (5-HT3R) antagonist granisetron evoked a dose-dependent attenuation of both 5-HT- and T-2 toxin-induced anorectic responses. Pretreatment with neurokinin-1 receptor (NK-1R) antagonist Emend® dose-dependently attenuated both SP- and T-2 toxin-induced anorectic responses. To summarize, the results suggest that both 5-HT and SP play important roles in anorexia induction by T-2 toxin. 5-HT is more potent and long-acting than SP in this response.

1. Introduction

T-2 toxin, a type A trichothecene mycotoxin produced by *Fusarium langsethiae* and *sporotrichioides*, contaminates cereal grains including wheat, barley, maize, rice and corn (Pestka, 2010a). Because these trichothecenes are resistant to food processing and milling, they can enter into food chain (Jackson and Bullerman, 1999). Ingestion of grains contaminated with trichothecene mycotoxins have been etiologically associated with large outbreaks of non-infectious gastroenteritis in several countries including United States, China, Russia, Japan, Korea, and India (Pestka, 2010b). T-2 toxin is considered to elicit the most potent toxicity among trichothecene mycotoxins (Devreese et al., 2013). Toxicosis of this toxin have been reported in both animals and human, with a spectrum of adverse effects including anorexia, emesis, growth retardation, immunosuppression and neuroendocrine changes (Li et al., 2011; Edwards et al., 2009; Liu et al., 2016). T-2 toxin's capacity to elicit anorectic response is of particular concern from the perspective of human and animal health (JECFA, 2002). This adverse response was observed in several animal species including rat, cat and rabbit (Fairhurst et al., 1987; Ferreras et al., 2013; Lutsky and Mor, 1981; Sato et al., 1975). Moreover, T-2 toxin was documented to cause food poisoning outbreak in China with a characteristic onset of anorexia in 97 persons that consumed contaminated rice by this toxin (Wang et al., 1993).

Appetite is regulated by various central and peripheral modulators via influencing the balance of anorexigenic and orexigenic signaling (Schwartz, 2006). Anorexia is an abnormal eating behavior and usually serves as a protective reflex against food poisoning by decreasing food intake. Trichothecene mycotoxins have the capacity of regulating the expression of anorexigenic and orexigenic factors within the brain. Deoxynivalenol (DON), a common type B trichothecene, has been reported to induce robust anorectic response (Pestka and Zhou, 2000). The possible mechanisms for DON-induced anorexia involve in upregulating the central anorexigenic factors including the pro-opiomelanocortin (POMC), melanocortin 4 receptor (MC4R) and cocaine amphetamine-regulated transcript (CART) in mouse hypothalamic neurons (Girardet et al., 2011a, b). T-2 toxin was observed to cause marked anorectic response by decreasing the expression of central orexigenic molecule neuropeptide Y (NPY) within mouse brain (Gaigé et al., 2014). Importantly, neurotransmitters secreted by enterochromaffin

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(EC) cells located in the gastrointestinal (GI) tract and central nervous system (CNS), appear to be critical upstream modulator for regulation of anorexigenic and orexigenic signaling (Anderberg et al., 2017; Krowicki and Hornby, 2000; Vlaev et al., 2017).

5-hydroxytryptamine (5-HT, serotonin), a monoamine neurotransmitter primarily produced by EC cells within the GI tract and CNS, was reported to be an important anorexigenic factor (Grundy, 2008; Vlaev et al., 2017; Blundell, 1977). 5-HT plays contributor role in regulation of food consumption by elevating POMC and CART expression within the hypothalamic neurons (Garfield and Heisler, 2009; Miryala et al., 2011; Jean et al., 2007; Sohn et al., 2011). Trichothecene mycotoxins T-2 toxin and DON were observed to induce plasma elevation of 5-HT in mink, and this was associated with emesis induction by two toxins (Wu et al., 2013, 2016). Substance P (SP) is another neurotransmitter involving in appetite regulation and also secreted by EC cells located in the GI tract and CNS (Darmani et al., 2009; Dey et al., 2010; Li, 2007). SP was documented to regulate food consumption by upregulating POMC expression within the hypothalamic neurons (Jessop et al., 2000; Frisch et al., 2010). Although 5-HT and SP contribute to appetite regulation, the roles of these two neurotransmitters in anorexia induction by T-2 toxin remain unclear.

The objective of this study was to test the hypothesis that neurotransmitters 5-HT and SP mediate T-2 toxin-induced anorectic response. To address this hypothesis, we employed a nocturnal mouse anorexia bioassay to: (1) characterize the kinetics and dose response of T-2 toxin-induced plasma 5-HT and SP elevation, (2) characterize anorectic response induced by T-2 toxin, 5-HT and SP, (3) characterize the effects of 5-HT and SP receptor antagonists on T-2 toxin-induced anorectic response.

2. Materials and methods

2.1. Chemicals

T-2 toxin was obtained from Toronto Research Chemicals (Toronto, Ontario, CA) and purity (> 98%) was verified by thin layer chromatography. For exposure studies, T-2 toxin was dissolved in 1% dimethylsulfoxide (DMSO) in filter-sterilized phosphate buffered saline (PBS) and orally administered to mouse at 0, 0.01, 0.1, 0.5 and 1 mg/kg bw. The selection of T-2 toxin concentrations is based on our previous finished work (Zhang et al., 2018). Both 5-HT (Tocris Biosciences) and SP (R&D Systems, Inc) were prepared in PBS to provide IP injection at doses up to 0.25 and 0.5 mg/kg bw, respectively. The 5-HT$_3$R antagonist granisetron (Tocris Biosciences) was prepared in PBS and administered by oral gavage to mouse at doses of 0, 0.1 and 0.5 mg/kg bw. The NK-1R antagonist Emend® (Merck & Co, Inc) was dissolved in 1% DMSO in filter-sterilized PBS and orally administered to mouse at 0, 1 and 2 mg/kg bw. Doses of various pharmacologic agents were selected based on supplier recommendations, prior animal investigations (Yamamoto et al., 2014; Rodgers et al., 2007; Hasenöhrl et al., 1994) and preliminary studies in the mouse.

2.2. Animals

10–12 weeks old, female B6C3F1 mice (average weight = 20 ± 3 g) were obtained from the Beijing Vital River Laboratories and housed individually in polycarbonate cages in a room maintained at 21–24 °C and 40–55% relative humidity under a 12 h light (6:00–18:00 h)/dark (18:00–6:00 h) cycle. High fat diet (45 kcal% fat diets, Jiangsu Medicine Company, Yangzhou, China) put in 2 inch high glass jars was employed for feeding bioassay and sifted aspen chips used for bedding. All experiments and protocols used in this study were approved by the Nanjing Agricultural University Institutional Animal Care and Use Committee (Certification No.: SYXK (Su) 2011-0036).

2.3. 5-HT and SP studies

Mice were acclimated to environment and pelleted high-fat diet for 1 week after arriving and then randomly divided into different groups according to body weight. On the day of experiment, mice were fasted from 10:00 h to 18:00 h and water provided ad lib. For kinetic studies, groups of mice (n = 6) were administered with 0 or 1 mg/kg bw T-2 toxin in 100 μl by oral gavage using a sterile 22 G 1.5 in. disposable feeding tube. Mice were sacrificed at 0, 0.5, 2, 6 and 24 h after exposure to T-2 toxin. For dose response studies, groups of mice (n = 6) were orally administered with 0, 0.01, 0.1, 0.5 and 1 mg/kg bw T-2 toxin in 100 μl, and sacrificed at 2 and 24 h after exposure.

At experiment termination, mice were anesthetized by IP injection with 100 μl 56 mg/ml sodium pentobarbital. Blood was collected with sterile syringes containing 20 μl 1% (vol/vol) EDTA (pH 7.5) from the inferior vena cava and transferred to EDTA-coated tubes. Plasma was separated from blood by centrifugation at 3500 × *g* for 10 min at 4 °C and frozen at −80 °C for neurotransmitters analyses. Plasma concentrations of 5-HT and SP were analyzed by enzyme-linked immunosorbent assay kit from ENZO Life Sciences (New York, USA) for 5-HT and enzyme immunoassay kit from Phoenix Pharmaceuticals (Burlingame, CA) for SP (human-, rat- and mouse-specific).

2.4. T-2 toxin and neurotransmitters-induced anorexia studies

Groups of mice (n = 6) were fasted from 10:00 to 18:00 h and water provided ad lib. To assess kinetic of anorexia induced by T-2 toxin, mice were orally administered with 1 mg/kg bw T-2 toxin in 100 μl and food intake was measured at 0.5, 1, 2, 3, 6 and 24 h post exposure. To determine dose response of anorexia induced by T-2 toxin, mice were orally administered with 0, 0.01, 0.1, 0.5 and 1 mg/kg bw T-2 toxin in 100 μl, and food intake was measured at 2 and 24 h after exposure. To assess neurotransmitters-induced anorexia, mice were IP injected with 100 μl 5-HT or SP at 0.25 or 0.5 mg/kg bw, respectively, and food intake was measured at 0.5, 1, 2, 3 and 6 h post exposure.

2.5. Neurotransmitter receptor studies

To establish that 5-HT$_3$R antagonist granisetron or NK-1R antagonist Emend® could attenuate 5-HT or SP-induced anorexia, 100 μl of each antagonist was given by oral gavage at 0, 0.1 and 0.5 mg/kg bw or 0, 1 and 2 mg/kg bw doses, respectively. After 30 min, mice were given an IP injection of 5-HT or SP at 0.25 or 0.5 mg/kg bw in 100 μl, respectively. Control groups were first gavaged with either vehicle (1% DMSO) or antagonists (0.5 mg/kg bw granisetron or 2 mg/kg bw aprepitant), then IP injected with PBS. Food intake was measured at 0.5, 2 and 6 h post-treatment. To ascertain the potential role of 5-HT or SP in T-2 toxin-induced anorexia, mice were gavaged with 100 μl of antagonist (0, 0.1 and 0.5 mg/kg bw granisetron or 0, 1 and 2 mg/kg bw aprepitant) 30 min before oral exposure to 100 μl of T-2 toxin at 1 mg/kg bw. Controls and food intake measurement time points were the same as for the neurotransmitter study.

2.6. Statistics

Data were analyzed using SigmaPlot 11 for Windows (Jandel Scientific; San Rafael, CA). Means were significantly different at $p < 0.05$. A two-way ANOVA using Holm-Sidak method was used to assess significant differences in 5-HT and SP concentrations in plasma over time. A one-way ANOVA using Holm-Sidak method was used to analyze statistical significance for multiple groups. Two-way repeated ANOVA (one-factor) using Holm-Sidak Method was used to analyze significant differences in food consumption as compared to the control over time. A one-way ANOVA using Student-Newman-Keuls method was used to analyze significant differences between treatment groups in food consumption for 5-HT$_3$ and NK-1 receptor antagonist study.

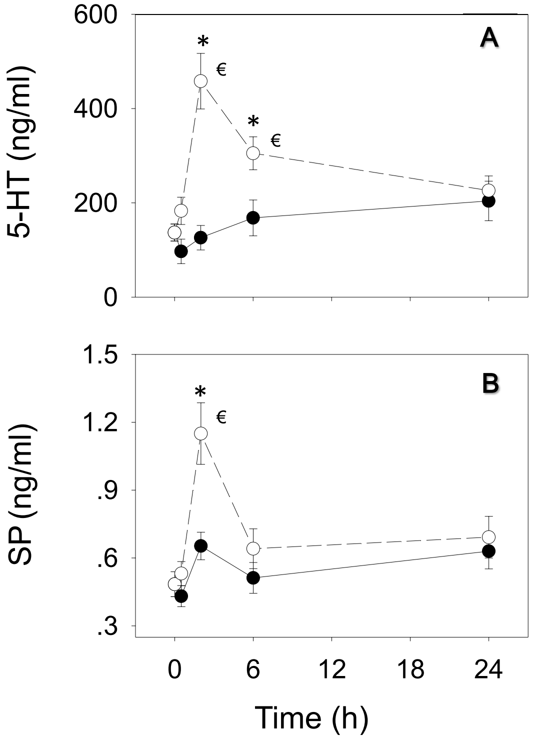

Fig. 1. Kinetics of T-2 toxin-induced plasma (A) 5-HT and (B) SP concentrations. Mice were orally gavaged with either PBS (solid lines) or 1 mg/kg bw T-2 toxin (broken lines). Data represent mean ± SEM (n = 6/gp). Two-way ANOVA using Holm-Sidak method was used to assess significant differences in kinetics of 5-HT and SP concentrations in plasma as compared to the control. Symbols: * indicates difference in plasma 5-HT or SP concentration relative to the control at specific time point ($p < 0.05$) and € indicates difference in plasma 5-HT or SP concentration relative to the 0 h time point ($p < 0.05$).

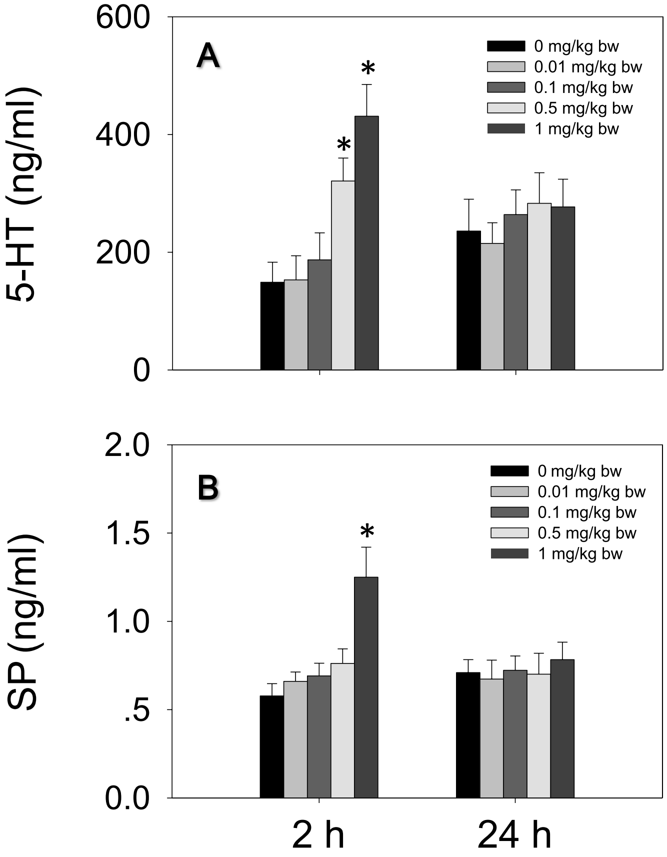

Fig. 2. Dose responses of T-2 toxin-induced plasma (A) 5-HT and (B) SP concentrations. Mice were orally gavaged with 0, 0.01, 0.1, 0.5 and 1 mg/kg bw T-2 toxin. Data represent mean ± SEM (n = 6/gp). A one-way ANOVA using Holm-Sidak method was used to analyze statistical significance for multiple groups. Symbols: * indicates difference in plasma 5-HT or SP concentration relative to the control ($p < 0.05$).

3. Results

3.1. T-2 toxin evoked plasma 5-HT and SP elevation

The kinetics of T-2 toxin-induced plasma 5-HT and SP elevation were assessed. 5-HT was significantly elevated by oral T-2 toxin treatment after 2 and 6 h, but recovered to basal level by 24 h (Fig. 1A). SP was significantly increased only at 2 h following oral exposure to T-2 toxin at 1 mg/kg bw (Fig. 1B).

The effects of T-2 toxin at 0, 0.01, 0.1, 0.5 and 1 mg/kg bw doses on plasma 5-HT and SP were assessed. Oral administration of 0.5 and 1 mg/kg bw T-2 toxin markedly elevated plasma 5-HT relative to the vehicle control value at 2 h and this elevation was returned to basal level evident after 24 h (Fig. 2A). While, the 0.01 and 0.1 mg/kg bw dose had no effect. SP was significantly increased at 2 h and returned to basal level at 24 h after oral exposure to 1 mg/kg bw T-2 toxin (Fig. 2B). While, the 0.01, 0.1 and 0.5 mg/kg bw doses had no effect.

3.2. T-2 toxin, 5-HT and SP induce anorectic response

Exposure to T-2 toxin, exogenous 5-HT and SP on anorexia induction were assessed. 1 mg/kg bw T-2 toxin caused 79, 76, 83, 68 and 53% reduction in cumulative food intake at 0.5, 1, 2, 3 and 6 h, respectively, with no differences being observed after 24 h (Fig. 3A). Mice orally administered with T-2 toxin at 0.1, 0.5 and 1 mg/kg bw consumed 48, 61 and 63% less food after 2 h compared to the control, respectively, while 0.01 mg/kg bw had no effect (Fig. 3B). After 24 h post-exposure, no significant reductions were observed in cumulative food intake between T-2 toxin- and vehicle-treated groups.

Dramatic reductions in cumulative food intake were observed for 5-HT treatment after 0.5 (65%), 1 (70%), 2 (54%) and 3 (43%) h, respectively, with no differences being observed beginning at 6 h (Fig. 4A). Cumulative food intake were reduced in mice exposed to SP by 51, 65 and 60% at 0.5, 1 and 2 h, respectively. Beginning at 3 h, no differences between control and SP group were observed (Fig. 4B).

3.3. T-2 toxin, 5-HT and SP-induced anorectic responses are attenuated by 5-HT$_3$R and NK-1R antagonists

The effects of blocking the 5-HT$_3$R in 5-HT- and T-2 toxin-induced anorexia were evaluated. IP administration of 5-HT at 0.25 mg/kg bw significantly decreased food intake at 0.5 and 2 h (Fig. 5A). After 6 h, no differences were observed when compared to control. 5-HT-induced anorectic response was dose-dependently attenuated with the 5-HT$_3$R antagonist granisetron at 0.5 and 2 h post-treatment. Exposure to the highest dose (0.5 mg/kg bw) of granisetron alone had no effect on food intake therefore excluding the possibility of functional antagonism. T-2 toxin evoked significant reduction in food intake relative to control (Fig. 5B). Following pretreatment of granisetron at 0.1 mg/kg bw, mice exposed to T-2 toxin consumed 15, 27 and 30% more food at 0.5, 2 and 6 h, respectively. Mice receiving 0.5 mg/kg bw granisetron consumed 33, 36 and 32% more food at 0.5, 2 and 6 h than T-2 toxin alone, respectively.

The effects of NK-1R antagonist on both SP- and T-2 toxin-induced anorexia were assessed. SP induced a significant reduction in food intake at 0.5 and 2 h, respectively, with no differences being observed after 6 h following IP exposure (Fig. 6A). This effect was dose-dependently attenuated with NK-1R antagonist Emend®. In order to exclude

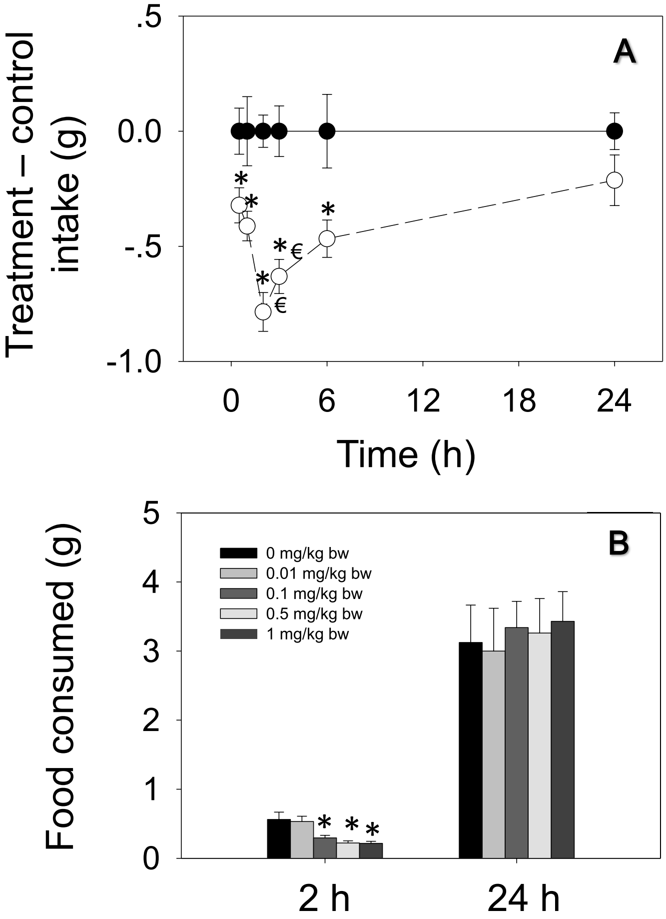

Fig. 3. (A) Kinetic and (B) dose response of T-2 toxin-induced food refusal. (A) Mice were orally administered with either 1% DMSO (solid lines) or 1 mg/kg bw T-2 toxin (broken lines) at initiation of the dark period, and food consumption was measured at various time points. Data represent mean ± SEM (n = 6/gp). Two-way repeated ANOVA (one factor) using Holm-Sidak Method was used to analyze significant differences in food consumption as compared to the control. Symbols: * indicates a statistically significant difference relative to the control at that specific time point ($p < 0.05$). $^€$ indicates a significant difference in cumulative food consumption relative to the 0.5 h time point within a given dose ($p < 0.05$). (B) Mice were orally gavaged with 0, 0.01, 0.1, 0.5 and 1 mg/kg bw T-2 toxin. Food intake was measured at 2 and 24 h after exposure. Data represent mean ± SEM (n = 6/gp). A one-way ANOVA using Holm-Sidak method was used to analyze statistical significance for multiple groups. Symbols: * indicates difference in food intake relative to the control ($p < 0.05$).

the possibility of functional antagonism, mice were treated with the highest dose (2 mg/kg bw) Emend® alone and no effect was observed on food intake. Oral administration of T-2 toxin markedly reduced food intake relative to control (Fig. 6B). Mice pretreated with 1 mg/kg bw Emend® consumed 20 and 24% more food at 2 and 6 h than T-2 toxin alone, respectively, and no difference was observed when pretreated with Emend® at 0.5 h. Following pretreatment of Emend® at 2 mg/kg bw, mice exposed to T-2 toxin consumed 21, 35 and 36% more food at 0.5, 2 and 6 h, respectively.

4. Discussion

T-2 toxin-induced anorectic response is a hallmark effect of trichothecene mycotoxin food poisoning and a major concern from the perspective of human and animal health. Prior investigations have revealed that gut satiety hormone involves in anorexia induction by T-2 toxin (Zhang et al., 2017a, b). However, much less is known about the role of neurotransmitter in this response. This study is novel because it is the first to systematically demonstrate the potential roles for two major neurotransmitter 5-HT and SP in mediating T-2 toxin-induced anorectic response. The results revealed that dose responses and kinetics of plasma 5-HT and SP secretion induced by T-2 toxin correspond to its anorectic action. In addition, like T-2 toxin, administration of exogenous 5-HT and SP induce rapid anorectic response. Both 5-HT$_3$R and NK-1R antagonism dose-dependently attenuated T-2 toxin-induced anorectic responses, suggesting T-2 toxin's anorectic response might partially be neurotransmitter-driven.

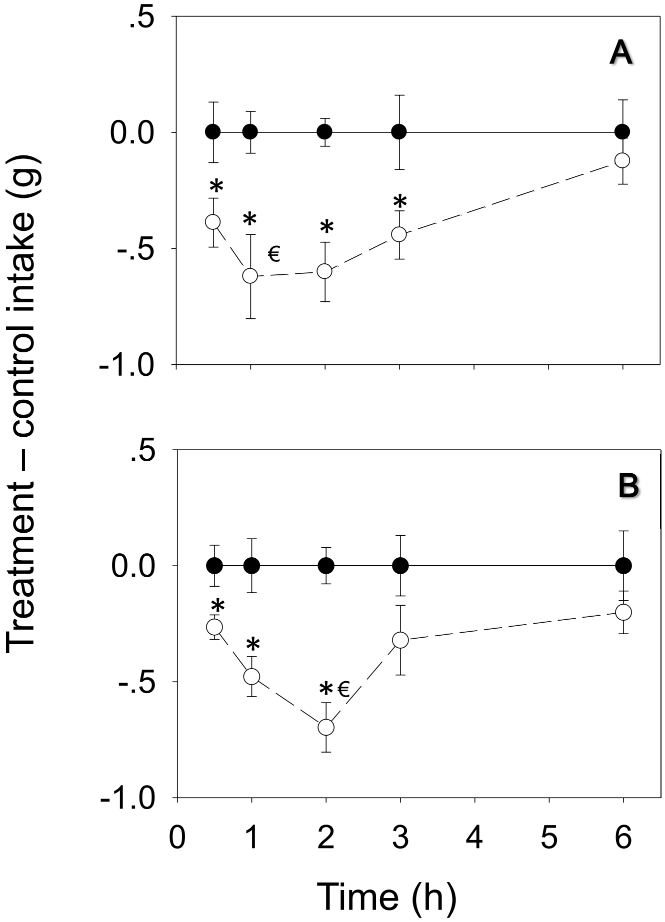

Fig. 4. Kinetics of (A) 5-HT- and (B) SP-induced anorectic response. Mice were IP administered with 0.25 or 0.5 mg/kg bw 5-HT or SP at initiation of the dark period, and food intake was measured at various time points. Data analyzed as described in Fig. 3 legend.

Our results for T-2 toxin's anorectic effect are highly consistent with previous investigation (Zhang et al., 2018). In that paper, T-2 toxin concentrations from 0 to 1 mg/kg bw was used to evaluate T-2 toxin's anorectic effect. The no observed adverse effect levels (NOAELs) and lowest observed adverse effect levels (LOAELs) were 0.01 and 0.1 mg/kg bw. Here, we used the same doses to explore the role of neurotransmitters 5-HT and SP in T-2 toxin-induced anorexia. Elicitation of anorexia by T-2 toxin corresponded to elevated plasma concentrations of 5-HT and SP. Although 0.1 mg/kg bw T-2 toxin had no effect on 5-HT and SP, the possible reason might be other anorexigenic factors involved in anorexia induction by T-2 toxin at this dose. For instance, gut satiety hormones glucose-dependent insulinotropic polypeptide (GIP), peptide YY$_{3-36}$ (PYY$_{3-36}$) and glucagon-like peptide-1(7–36) amide (GLP-1) are documented to mediate anorexia and increased by T-2 toxin at 0.1 mg/kg bw (Wu et al., 2018; Sheng et al., 2018). Moreover, neurotransmitters also appear to be important targets of T-2 toxin and play an important upstream role in appetite regulation (Anderberg

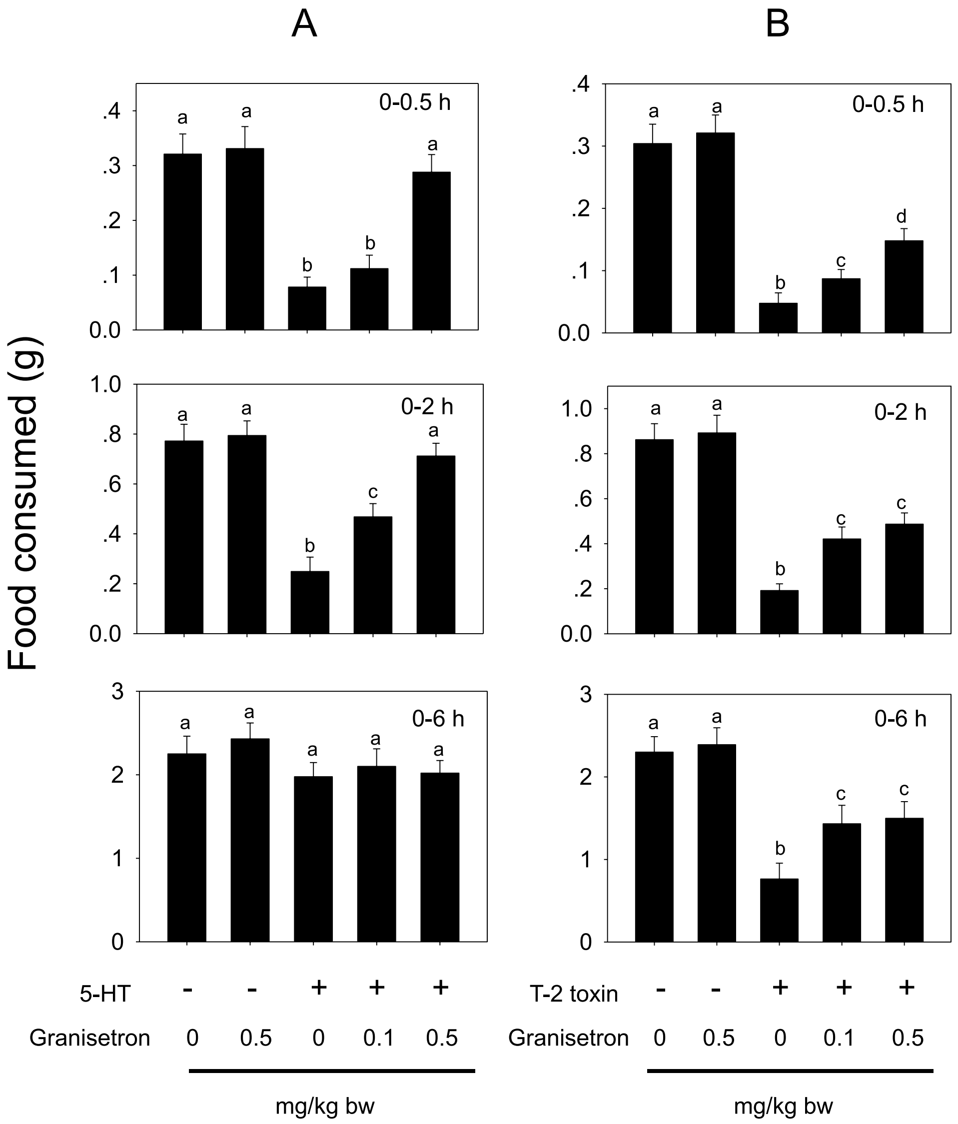

Fig. 5. 5-HT$_3$ receptor antagonist granisetron inhibits (A) 5-HT- and (B) T-2 toxin-induced anorectic response. Mice were pretreated with 0, 0.1 and 0.5 mg/kg bw granisetron and 30 min later treated with PBS, 0.25 mg/kg bw 5-HT or 1 mg/kg bw T-2 toxin. Food intake was then measured at 0.5 h, 2 h, and 6 h post administration. Data are mean ± SEM (n = 6/gp) and analyzed by one way ANOVA using Student-Newman-Keuls Method. Bars without the same letter are significantly different ($p < 0.05$).

et al., 2017; Krowicki and Hornby, 2000). 5-HT and SP are two of the most studied neurotransmitters with known anorectic effects through peripheral and central mechanisms (Blundell and Halford, 1998; Dib, 1999). 5-HT is potent in its ability to cause rats to refuse food following IP and intracerebroventricular administration (Stevens and Edwards, 1996; Leibowitz et al., 1993). While, both peripheral and intracerebroventricular injection of SP are observed to evoke food refusal in rats (Holzer, 1985; Hasenöhrl et al., 1994; Dib, 1999). As demonstrated here, IP administration of 5-HT and SP induced marked anorectic responses, as evidenced by immediate anorexia in 0.5 h and lasting 3–6 h. Our findings are consistent with previous reports, demonstrating 5-HT and SP mediate anorectic responses.

When 5-HT and SP is secreted by EC cells in GI tract, they can act in a paracrine manner following complex physiological processes involving: binding specific receptors located on the abdominal vagal afferents, → signaling satiation via brain-gut axis, → changing expression of anorexigenic and orexigenic signaling molecules within the hypothalamus, → altered regulation of appetite and resulted in anorexia (De and Schreiber, 2000; Krowicki and Hornby, 2000). In addition to be produced by EC cells in GI tract, 5-HT and SP are also secreted within CNS including various brain regions, suggesting these neurotransmitters might act in an endocrine fashion on CNS (Jean et al., 2007; Darmani et al., 2009; Dey et al., 2010). It is notable that another important trichothecene, DON is reported to rapidly distribute into mouse brain and evokes c-Fos and POMC expression in the circumventricular organs of the brain and surrounding regions (Pestka et al., 2008; Girardet et al., 2011a, b). Furthermore, DON has been shown to activate central anorectic pathways by activating hypothalamic neurons of NUCB2/nesfatin-1 which has the potency to elicit anorectic effect (Gaigé et al., 2013; Oh-I et al., 2006). Importantly, 5-HT was elevated in the rat cerebellum and hypothalamus following oral administration of DON (Fitzpatrick et al., 1988). Similar to DON, T-2 toxin can also induce c-Fos expression in key brainstem and stimulate NUCB2/nesfatin-1 neurons in the hypothalamus resulted in anorexia (Gaigé et al., 2014). It is possible that T-2 toxin directly target the CNS to modulate anorectic neurocircuitry by stimulating 5-HT and SP secretion in the brain.

Our findings here demonstrated that use of 5-HT$_3$R antagonist inhibited anorexia induction by T-2 toxin. 5-HT elicits its various functions including regulation of aggression, anxiety, appetite, cognition

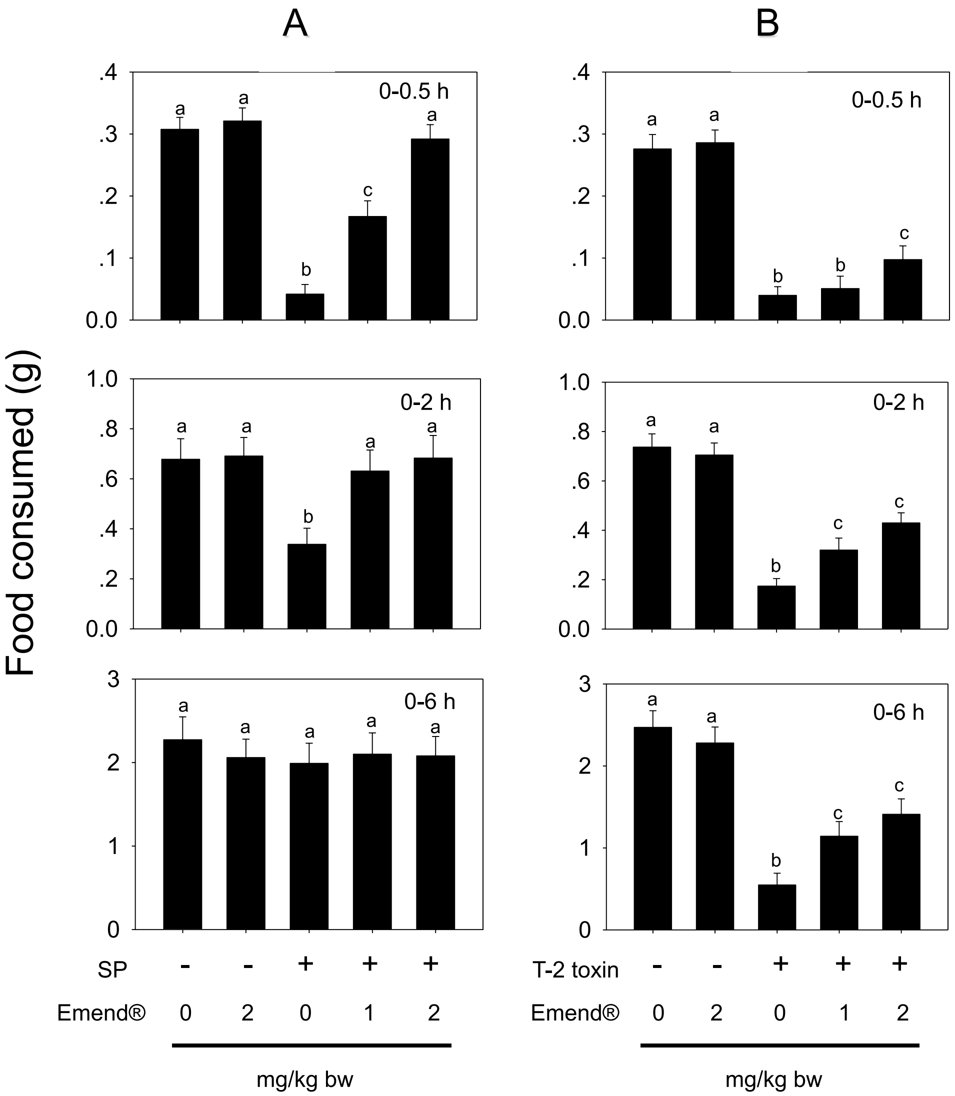

Fig. 6. NK-1 receptor antagonist Emend® inhibits (A) SP- and (B) T-2 toxin-induced anorectic response. Mice were pretreated with 0, 1 and 2 mg/kg bw Emend® and 30 min later treated with 1% DMSO, 0.5 mg/kg bw SP or 1 mg/kg bw T-2 toxin. Food intake was then measured at 0.5 h, 2 h, and 6 h post administration. Data are mean ± SEM (n = 6/gp) and analyzed by one way ANOVA using Student-Newman-Keuls Method. Bars without the same letter are significantly different ($p < 0.05$).

and emesis via binding several receptor subtypes including 5-HT$_1$B, 5-HT$_2$, 5-HT$_2$C, and 5-HT$_3$ receptors (Nichols and Nichols, 2008). For instance, Bouwknecht and co-workers indicated that 5-HT$_{1B}$R knockout mice elicited marked increases in food intake compared with wild-type mice (Bouwknecht et al., 2001). Activating 5-HT$_{2c}$R has been shown to induce food intake reduction in rats (De and Schreiber, 2000). In prior studies, the contributions of 5-HT in emesis induction by T-2 toxin and DON were investigated in mink (Wu et al., 2013, 2016). T-2 toxin- and DON-induced plasma 5-HT secretion corresponded to their emetic action. In support of this contention, blocking 5-HT$_3$R attenuates DON-induced emesis in mink and pig (Wu et al., 2013; Prelusky and Trenholm, 1993). Moreover, delayed gastric emptying, which is considered as a surrogate for emesis, was observed in rodents following DON exposure via 5-HT$_3$R (Fioramonti et al., 1993). Another type A trichothecene mycotoxin diacetoxyscirpinol (DAS) elicited significant anorectic response similar to T-2 toxin (Zhang et al., 2017a, b). Andrews and co-workers indicated that emesis induction by DAS could be attenuated by abdominal vagotomy or blocking 5-HT$_3$R in ferret (Andrews et al., 1990), which is further consistent with our finding. Therefore, it could speculated that 5-HT mediates T-2 toxin-induced anorectic response by activating 5-HT$_3$R similar to DON and DAS.

It was particularly notable that, for the first time, T-2 toxin-induced anorexia was attenuated by NK-1R antagonist. Neurokinin receptors belong to the class I G-protein coupled receptor family and contain seven transmembrane loops, including three main classes NK-1R, NK-2R and NK-3R (Maggi, 1995; Datar et al., 2004). The highest affinity receptor for SP is NK-1R and SP elicits its various biologic functions including anxiety, appetite, pain, inflammation and emesis through this receptor (Bassi et al., 2017). Cisplatin, a cancer chemotherapeutic agent, has been shown to evoke SP secretion as well as emetic and anorectic responses (Hesketh et al., 2003; Yamamoto et al., 2014). Use of the NK-1R inhibitor, Emend® can attenuate emetic and anorectic response induction by this chemotherapeutic agent. Interestingly, cisplatin also has the potency to induce 5-HT secretion as well as emetic and anorectic responses similar to DON and T-2 toxin (Percie du Sert et al., 2011; Castejon et al., 1999). Blocking 5-HT$_3$R can suppress emetic and anorectic responses induced by not only cisplatin but also DON (Wu et al., 2013; Fioramonti et al., 1993; Castejon et al., 1999; Takeda et al., 2008). Therefore, the observation here that Emend® can attenuated T-2 toxin-induced anorexia, suggest that as found for cisplatin, SP might contribute to anorexia induction by T-2 toxin via NK-1R.

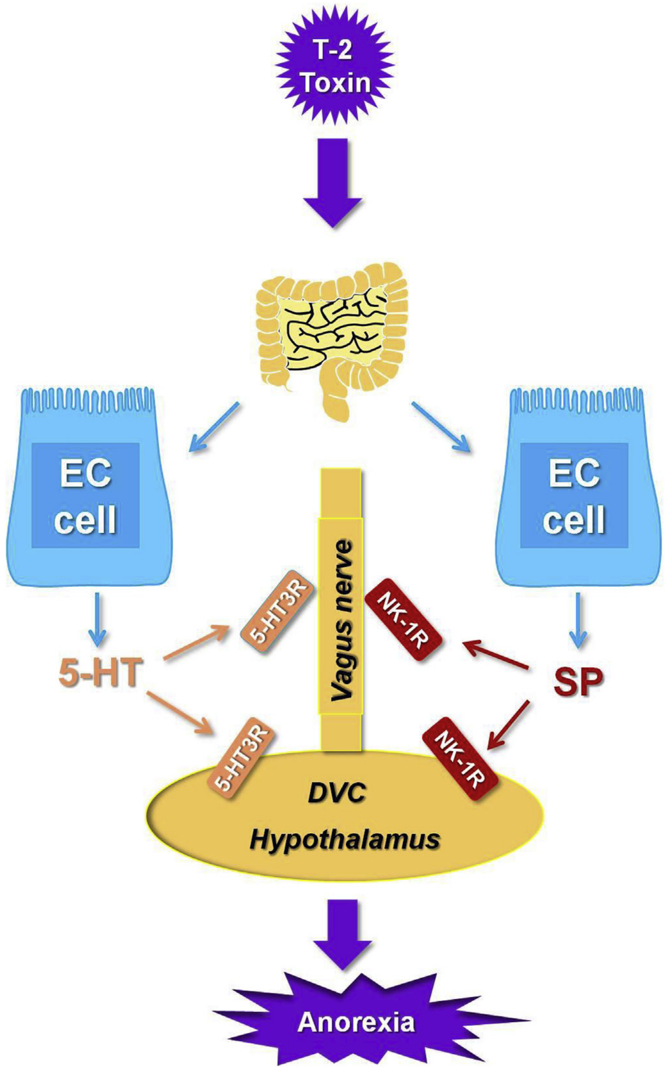

Fig. 7. Putative role of 5-HT and SP in T-2 toxin-induced anorectic response. The results presented here suggest that T-2 toxin might act on the EEC to release 5-HT and SP. 5-HT_3R and NK-1R located in the peripheral and central nervous systems are activated sequentially to contribute to anorexia.

It is important to note that anorectic response occurred earlier than the elevation of 5-HT and SP following administration of T-2 toxin, suggesting that other anorexigenic modulators might be involved in the beginning of anorexia. In previous studies, we have investigated the roles of gut satiety hormones cholecystokinin (CCK), GIP, PYY_{3-36} and GLP-1 in anorexia induction by T-2 toxin (Zhang et al., 2017a, b; Wu et al., 2018; Sheng et al., 2018). Elevations of plasma GIP, PYY_{3-36} and GLP-1 were observed within 0.5 h, which coincided with the occurrence of anorectic response. Furthermore, these satiety hormones also contribute to DON and other type B trichothecenes-induced anorexia (Wu et al., 2014a; Jia et al., 2017). In addition to gut satiety hormone, the proinflammatory cytokines also contribute to anorexia induction (Plata-Salaman, 1998). The trichothecenes including T-2 toxin are well-known to rapidly induce release of proinflammatory cytokines including interleukin-1β (IL-1β), interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) (Wu et al., 2014b; Agrawal et al., 2012; Wu and Zhang, 2014). It might be speculated that T-2 toxin-induced anorectic response might be partially mediated by these cytokines.

In summary, the results provided herein suggest that T-2 toxin elicits 5-HT- and SP-driven anorectic responses by activating 5-HT_3R and NK-1R as depicted in Fig. 7. In a prior investigation, we have revealed that DON-induced 5-HT secretion was resulted from activation of calcium-sensing receptor (CaSR), a G-coupled protein receptor, and transient receptor potential ankyrin-1 (TRPA1), a TRP channel (Wu et al., 2017). Future investigations should focus on how T-2 toxin and other trichothecenes act on primary EC to elicit 5-HT and SP exocytosis as well as their linkages to subsequent anorectic response. From a public health perspective, research such as this will improve our understanding of how T-2 toxin evoke anorexia as well as other adverse effects in animal and human, and formulate strategies to prevent food poisoning for this toxin.

Conflicts of interest

The authors declare that there are no conflicts of interest.

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