Ecotoxicology and Environmental Safety 153 (2018) 181–187

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

Potential roles for glucagon-like peptide-1_{7-36} amide and cholecystokinin in anorectic response to the trichothecene mycotoxin T-2 toxin

Wenda Wu[a,b], Kun Sheng[a], Xinglian Xu[b], Haibin Zhang[a,b,*], Guanghong Zhou[b,*]

[a] College of Veterinary Medicine, Nanjing Agricultural University, Nanjing 210095, PR China
[b] Key Laboratory of Meat Processing, Key Lab of Meat Processing and Quality Control, Collaborative Innovation Center of Meat Production and Processing, Quality and Safety Control, National Center of Meat Quality and Safety Control, College of Food Science and Technology, Nanjing Agricultural University, Nanjing 210095, PR China

ARTICLE INFO

Keywords:
Mycotoxin
T-2 toxin
Anorexia
Gut satiety hormone
Glucagon-like peptide-1_{7-36} amide
Cholecystokinin

ABSTRACT

Anorexia is a hallmark of animal and human exposed to T-2 toxin, a most poisonous trichothecene mycotoxins contaminating various cereal grains including wheat, corn and barley. Although this adverse effect has been well characterized in several animal species, the underlying mechanisms are unclear. The goal for this study was to elucidate the roles of two gut satiety hormones, glucagon-like peptide-1_{7-36} amide (GLP-1) and cholecystokinin (CCK) in T-2 toxin-evoked anorectic response using a mouse anorexia bioassay. Elevations of plasma GLP-1 and CCK significantly corresponded to anorexia induction by T-2 toxin. Direct administration of exogenous GLP-1 and CCK markedly evoked anorectic responses similar to T-2 toxin. The GLP-1 receptor (GLP-1R) antagonist Exendin9-39 dose-dependently cause attenuation of both GLP-1- and T-2 toxin-induced anorectic responses. Pretreatment with the CCK1 receptor (CCK1R) antagonist SR 27897 and CCK2 receptor (CCK2R) antagonist L-365,260 attenuated anorexia induction by both CCK- and T-2 toxin in a dose dependent manner. Taken together, our findings suggest that both GLP-1 and CCK play contributory roles in T-2 toxin-induced anorexia.

1. Introduction

Produced by *Fusarium*, the trichothecenes are a large group of chemical structurally-related mycotoxins that frequently contaminate cereal grains including rice, maize, barley and oats (Gaigé et al., 2014). Belonging to the type A trichothecenes, T-2 toxin is considered to elicit the most potent toxicity among trichothecenes (Devreese et al., 2013). Adverse effects of this toxin include anorexia, emesis, growth retardation and neuroendocrine changes (Li et al., 2011; Edwards et al., 2009). From animal and human health perspective, T-2 toxin-induced anorexia is of particular concern. This adverse effect was observed in several animal species exposed to T-2 toxin (Fairhurst et al., 1987; Ferreras et al., 2013). Despite the importance of anorexia induction by T-2 toxin, the underlying mechanisms for this adverse effect remain unclear.

Appetite regulation is a complex physiological processes involving various anorexigenic and orexigenic modulators (Schwartz, 2006). Deoxynivalenol (DON), a common type B trichothecene, has been reported to evoke robust anorectic response (Forsell et al., 1986), and possible mechanisms were involved in upregulating the central anorexigenic factors including the cocaine amphetamine-regulated transcript (CART), pro-opiomelanocortin (POMC) and melanocortin 4 receptor (MC4R) in mouse hypothalamic neurons (Girardet et al., 2011a, 2011b). T-2 toxin-induced anorectic response was related to decreasing the expression of central orexigenic molecule neuropeptide Y (NPY) within mouse brain (Gaigé et al., 2014). A possible upstream modulator for regulation of anorexigenic and orexigenic signaling is gut satiety hormone. These hormones are produced by enteroendocrine cells (EEC) in the gastrointestinal (GI) tract or central nervous system (CNS) and regulates appetite through gut-brain axis (Moran-Ramos et al., 2012). Glucagon-like peptide-1_{7-36} amide (GLP-1) and cholecystokinin (CCK) are two common gut satiety hormones which are reported to be important anorexigenic factors (Steinert et al., 2013). GLP-1 is a 30 amino acid poly peptide produced by L cells in small and large intestine (Williams et al., 2009). While, CCK (a 27 amino acid poly peptide) was the first reported gut satiety hormone secreted from I cells in small intestine (Strader and Woods, 2005). Both peripheral and central administration of two hormones have the potency to evoke marked anorectic response (Sanghee et al., 2008; Williams et al., 2006; Zhang et al., 1986). It was well demonstrated that mechanisms of anorexia induction by GLP-1 and CCK were associated with influencing the anorexigenic and orexigenic signaling in the hypothalamus. ICV infusion of GLP-1 could up-regulate anorexigenic factors (POMC and CART) and down-regulate orexigenic molecules (NPY and agouti-related protein [AgRP]) within the brain in fasted rodents (Seo et al., 2008). CCK has

* Corresponding author.
E-mail addresses: zhhbnau@163.com (H. Zhang), guanghong.zhou@hotmail.com (G. Zhou).

https://doi.org/10.1016/j.ecoenv.2018.02.003
Received 18 December 2017; Received in revised form 28 January 2018; Accepted 2 February 2018

been implicated in decreasing food consumption via increasing hypothalamus expression of CART, POMC and MC4R (Strader and Woods, 2005; de Lartigue et al., 2007).

Here, we propose a hypothesis that gut satiety hormones GLP-1 and CCK mediate anorectic response evoked by T-2 toxin. To address this, we characterize (1) T-2 toxin-, GLP-1- and CCK-induced anorectic response, (2) elevation of T-2 toxin-induced plasma GLP-1 and CCK, (3) blockade of GLP-1 and CCK receptors on T-2 toxin-induced anorectic response.

2. Materials and methods

2.1. Toxin and chemicals

T-2 toxin (> 98%) was purchased from Toronto Research Chemicals (Toronto, Ontario, CA) and was dissolved in 1% dimethylsulfoxide (DMSO) in phosphate buffered saline (PBS). GLP-1, CCK, SR 27897 and L-365,260 were obtained from Tocris Biosciences (Ellisville, MO). GLP-1, CCK and Exendin9-39 (Sigma-Aldrich, St. Louis, MO) were dissolved in PBS. SR 27897 and L-365,260 were prepared in 5% DMSO. Doses selection of various pharmacologic agents were based on supplier recommendations and prior investigations (Lotti et al., 1986; Wu et al., 2014; Blandizzi et al., 1999; Cano et al., 2003).

2.2. Animals

B6C3F1 mice (female, 10–12 weeks old) were gained from the Beijing Vital River Laboratories and housed individually in polycarbonate cages in a room under a 12 h light (6:00–18:00 h)/dark (18:00–6:00 h) cycle. The temperature and relative humidity maintained at 21–24 °C and 40–55%, respectively. All experiments were approved by the Nanjing Agricultural University Institutional Animal Care and Use Committee (Certification No.: SYXK (Su) 2011–0036).

2.3. T-2 toxin and satiety hormones-induced anorexia studies

The general experimental design was based on previously described protocols (Flannery et al., 2011). 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 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 assess satiety hormones-induced anorexia, mice were IP injected with 100 µl GLP-1 or CCK at 0.1 or 0.01 mg/kg bw, respectively, and food intake was measured at 0.5, 1, 2, 3 and 6 h post exposure.

2.4. GLP-1 and CCK studies

T-2 toxin-induced GLP-1 and CCK secretion were assessed using a prior developed protocol (Wu et al., 2014). For dose response studies, mice 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. For kinetic studies, mice were administered with 0 or 1 mg/kg bw T-2 toxin in 100 µl by oral gavage. Mice were sacrificed at 0, 0.5, 2, 6 and 24 h post exposure. The procedure for blood collection and plasma separation were based on previous study (Wu et al., 2014). Plasma GLP-1 and CCK were analyzed by enzyme immunoassay kits.

2.5. Satiety hormone receptor studies

To establish that GLP-1R antagonist Exendin9-39 or CCKR antagonist SR 27897 and L-365,260 could attenuate GLP-1 or CCK-induced anorexia, 100 µl of each antagonist was given by oral gavage at 0, 0.05 and 0.1 mg/kg bw or 0, 1 and 2.5 mg/kg bw doses, respectively. 30 min later, mice were IP injected GLP-1 or CCK at 0.1 or 0.01 mg/kg bw in 100 µl, respectively. Control groups were first gavaged with either vehicle (5% DMSO) or antagonists (0.1 mg/kg bw Exendin9-39 or 2.5 mg/kg bw SR 27897 and L-365,260), then IP injected with PBS. Food intake was measured at 0.5, 2 and 6 h post-treatment. To determine the roles of GLP-1 and CCK in T-2 toxin-induced anorexia, mice were gavaged with antagonist (0, 0.05 and 0.1 mg/kg bw Exendin9-39 or 0, 1 and 2.5 mg/kg bw SR 27897 and L-365,260) 30 min before orally exposed to 1 mg/kg bw T-2 toxin. Controls and food intake measurement time points were the same as for the satiety hormone study.

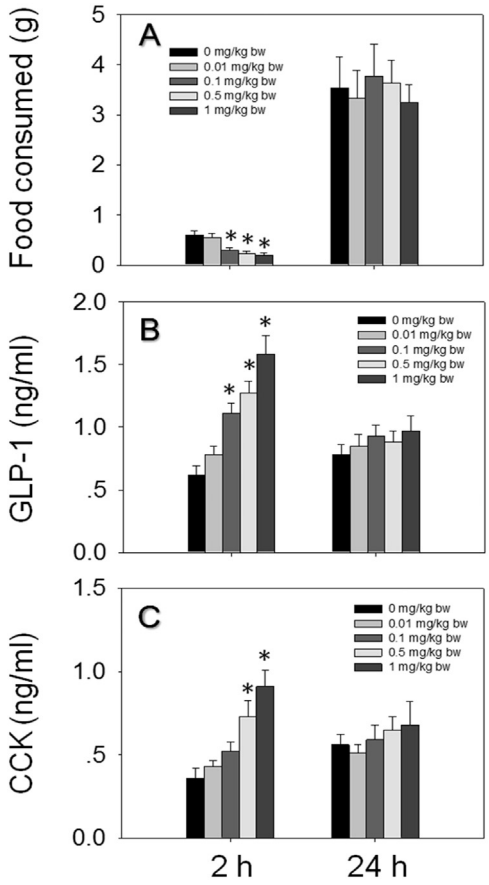

Fig. 1. Dose responses of T-2 toxin-induced (A) anorectic response, (B) GLP-1 and (C) CCK concentrations. Mice were orally gavaged with 0, 0.01, 0.1, 0.5 and 1 mg/kg bw T-2 toxin, food intake and satiety hormones were measured at 2 and 24 h after exposure. Data represent mean ± SEM (n = 6/gp). A one-way ANOVA using Holm-Sidak method was used to analyze statistical significance for multiple groups. Symbols: * indicates difference in food consumption relative to the control ($p < 0.05$).

2.6. Statistics

SigmaPlot 11 was used for data analysis and $p < 0.05$ was considered significantly different. Two-way ANOVA or two-way repeated ANOVA using Holm-Sidak method was used to assess significant differences in satiety hormone concentrations or food consumption over time, respectively. One-way ANOVA using Holm-Sidak method or Student-Newman-Keuls method was used to analyze statistical significance for multiple groups.

3. Results

3.1. T-2 toxin evoked plasma GLP-1 and CCK elevation corresponding to anorectic response

Mice orally administered with T-2 toxin at 0.1, 0.5 and 1 mg/kg bw