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Anorexic action of deoxynivalenol in hypothalamus and intestine

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ABSTRACT

Although deoxynivalenol (DON) suppresses food intake and subsequent weight gain, its contribution to anorexia mechanisms has not been fully clarified. Thus, we investigated the anorexic actions of DON in the hypothalamus and intestine, both organs related to appetite. When female B6C3F1 mice were orally exposed to different doses of DON, a drastic anorexic action was observed at a dose of 12.5 mg/kg body weight (bw) from 0 to 3 h after administration. Exposure to DON (12.5 mg/kg bw) for 3 h significantly increased the hypothalamic mRNA levels of anorexic pro-opiomelanocortin (POMC) and its downstream targets, including melanocortin 4 receptor, brain-derived neurotrophic factor, and tyrosine kinase receptor B; at the same time, orexigenic hormones were not affected. In addition, exposure to DON significantly elevated the hypothalamic mRNA levels of proinflammatory cytokines (IL-1β, TNF-α, and IL-6) and activated nuclear factor-kappa B (NF-κB), an upstream factor of POMC. These results suggest that DON-induced proinflammatory cytokines increased the POMC level via NF-κB activation. Moreover, exposure to DON significantly enhanced the gastrointestinal mRNA levels of anorexic cholecystokinin (CCK) and transient receptor potential ankyrin-1 channel (TRPA1), a possible target of DON; these findings suggest that DON induced anorexic action by increasing CCK production via TRPA1. Taken together, these results suggest that DON induces anorexic POMC, perhaps via NF-κB activation, by increasing proinflammatory cytokines in the hypothalamus and brings about CCK production, possibly through increasing intestinal TRPA1 expression, leading to anorexic actions.

1. Introduction

Deoxynivalenol (DON) is one of the trichothecene mycotoxins produced by the *Fusarium* species, which contaminate cereals such as wheat, barley, and corn (Pestka, 2010). DON occurs in environments with high temperature and humidity conditions and is grown worldwide. It is difficult to destroy DON by cooking and processing. It has been estimated that DON intake by humans is 0.8, 1.2, 1.4, 1.6, and 2.4 μg/kg body weight (bw)/d in Africa, Latin America, Europe, the Middle Far East, and East, respectively (Canady et al., 2001). Due to its frequent occurrence in grain-based foods, it is important to understand how DON affects animals and humans.

High doses of DON cause diarrhea, vomiting, emesis, and malaise, whereas low doses cause impaired body weight gain and diminished nutritional efficiency (Pestka, 2010). Suppression of weight gain in mice has been used as the metric for establishing the tolerable daily intake of DON (Canady et al., 2001). Several studies have suggested that DON impairs body weight increase and induces body weight loss by suppressing food intake. For example, DON intake of 0.25–1.0 mg/kg and 1.0 mg/kg for female and male rats, respectively, for approximately 9 weeks decreased their body weights via decreasing rat chow consumption (Arnold et al., 1986).

Abbreviations: AGRP, agouti-related peptide; BDNF, brain-derived neurotrophic factor; CART, cocaine-and amphetamine-regulated transcript; CaSR, calcium-sensing receptor; CCK, cholecystokinin; CRH, corticotrophin-releasing hormone; DON, deoxynivalenol; GHRL, ghrelin; IκBα, inhibitor kappa B alpha; IKK, IκB kinase; IL-1β, interleukin-1β; IL-6, interleukin-6; IP3, inositol trisphosphate; MC4R, melanocortin 4 receptor; NF-κB, nuclear factor-kappa B; NPY, neuropeptide Y; PBS, phosphate-buffered saline; POMC, pro-opiomelanocortin; PVDF, polyvinylidene fluoride; PYY, peptide YY; TBST, tris-buffered saline containing 0.05% (v/v) Tween 20; TNF-α, tumor necrosis factor-α; TrkB, tyrosine kinase receptor B; TRPA1, transient receptor potential ankyrin-1; TRPM5, transient receptor potential cation channel subfamily M member 5; SDS, sodium dodecyl sulfate; SE, standard error.

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DON consumption (0.35 and 1.3 mg/kg) resulted in reductions in food intake and body weight after 1 week in mice (Robbana-Barnat et al., 1987). In spite of these studies, the underlying mechanisms remain poorly understood.

Anorexic effects of DON are possibly mediated through the brain (Bonnet et al., 2012; Maresca, 2013; Lebrun et al., 2015). Since DON is detectable in the brain following oral administration (Pestka et al., 2008), the toxin has the potential to interfere with normal appetite regulation. Indeed, exposure to DON at 2.5 mg/kg bw significantly increased serotonin levels in the cerebellum and hypothalamus in rats 24 h after administration (Fitzpatrick et al., 1988a; 1988b). In addition, acute administration of DON (0.25 mg/kg bw) elevated the serotonin levels in the hypothalamus of swine (Prelusky et al., 1992). Thus, DON-induced serotonin in the brain could mediate feed refusal and subsequent body weight loss. Recently, Girardet et al. (2011a) demonstrated that the anorexic effect of DON results from inhibition of the central neuron networks involved in food intake regulation. In addition, oral administration of DON elevated the mRNA levels of anorexic hormones such as pro-opiomelanocortin (POMC) and cocaine- and amphetamine-regulated transcription (CART) in mice.

Appetite is also controlled by the gastrointestinal tract as well as by the hypothalamus. Therefore, DON may affect gastrointestinal hormones related to appetite (Maresca, 2013; Lebrun et al., 2015). Flannery et al. (2012) have shown that DON administration increased the plasma levels of anorexic hormones including peptide YY (PYY) and cholecystokinin (CCK). In particular, such satiety hormonal responses after DON exposure were higher in aged rather than in adult mice (Clark et al., 2015). Interestingly, Zhou and Pestka (2015) proposed that DON-induced CCK production is mediated by the calcium-sensing receptor (CaSR) and transient receptor potential ankyrin-1 channel (TRPA1) in STC-1 cells. However, information on the mechanism by which DON regulates anorexic hormones is limited. Additional information should be accumulated to more accurately understand how DON modifies food intake in animals and human.

Thus, in mice, we investigated the effect of DON intake on appetite-related hormone responses in the hypothalamus and gastrointestinal tract and their mechanisms. Our study suggests that DON induced anorexic POMC via nuclear factor-kappa B (NF-κB) activation by increasing the levels of proinflammatory cytokines in the hypothalamus and anorexic CCK production via increasing intestinal TRPA1 expression.

2. Methods

2.1. Experiment design

Six-week-old female B6C3F1 mice were purchased from CLEA Japan Inc. (Tokyo, Japan) and housed individually in polycarbonate cages at 23–25 °C in a temperature-controlled room with relative humidity (50%–56%) under a 12-h light/dark cycle (lights on 8:00 a.m.). Mice were acclimated for 1 week and then divided into different groups (n = 5–6) depending on the number and type of experiments. On the day of the experiment, mice were fasted from 12:00 to 20:00 and given water *ad libitum*. The mice were orally administered 0, 6.25, 12.5, or 25 mg/kg body weight (bw) of DON (Wako Pure Chemical Industries, Ltd, Osaka, Japan) in 200 μL phosphate-buffered saline (PBS) at 20:00. After DON consumption, food intake was measured at 3, 6, 12, 18, and 24 h.

In other experiments, 6-week-old female B6C3F1 mice (n = 5–6) consumed 0 or 12.5 mg/kg bw of DON in 200 μL PBS. All mice were dissected at 3 h after toxin exposure, under anesthesia with 30 mg/kg bw Somnopentyl (pentobarbital sodium; Kyoritsu Seiyaku Corporation, Tokyo, Japan). The hypothalamus was separated from the brain lavaged with physiological saline, put into RNA *later*® Solution (Thermo Fisher Scientific Inc., Waltham, MA USA) at 4 °C overnight, and then stored at −30 °C until analysis. The stomach and small intestine were washed with physiological saline, rapidly frozen in liquid nitrogen, and stored at −80 °C until analysis. All animal experiments were approved by the Animal Care and Research Ethics Committee of the Tokyo University of Agriculture.

2.2. RNA extraction and quantitative reverse transcription polymerase chain reaction (RT-PCR)

Total RNA was extracted using Trizol® Reagent (Thermo Fisher Scientific Inc.) and treated with DNase I (Promega Corporation, Madison, WI, USA). Reverse transcription was performed using a high-capacity cDNA reverse-transcription kit (Thermo Fisher Scientific Inc.). Real-time PCR was performed on ABI PRISM 7300 Sequence Detection System (Thermo Fisher Scientific Inc.) using primer and probe sets (TaqMan Gene Expression Assays, Thermo Fisher Scientific Inc.) and Taqman Universal PCR Master Mix (Toyobo Co., Osaka, Japan), according to the manufacturer's instructions. Several target genes were chosen: POMC, CART, neuropeptide Y (NPY), agouti-related peptide (AGRP), melanocortin 4 Receptor (MC4R), brain-derived neurotrophic factor (BDNF), tyrosine kinase receptor B (TrkB), corticotrophin-releasing hormone (CRH), ghrelin (GHRL), CCK, PYY, interleukin (IL)-1β, IL-6, tumor necrosis factor (TNF)-α, TRPA1, and transient receptor potential cation channel subfamily M member 5 (TRPM5). The mRNA level of the targets was represented as a value relative to the level of β2-microglobulin. PCR amplifications were performed in duplicate wells in the same 96-well plates under the following conditions: 1) 2 min at 50 °C and 10 min at 95 °C, 2) followed by 60 cycles of 15 s at 95 °C and 1 min at 60 °C.

2.3. Immunoblot analysis

Hypothalamic proteins were extracted with an RIPA buffer (0.02 M Tris–HCl (pH7.4) containing 0.15 M NaCl, 1 mM ethylenediaminetetraacetic acid, 1% NP 40, 0.1% sodium deoxycholate, 1% sodium dodecyl sulfate (SDS), 1% Protease Inhibitor Cocktail (Sigma–Aldrich St. Louis, MO, USA), and 10% 10X Phosphatase Inhibitor Cocktail (Roche Diagnostics Corp. Indianapolis, IN, USA). The total amount of lysate protein was measured with an *RC DC*™ protein assay reagent (Bio-Rad Laboratories Inc., Hercules, CA, USA). Equal amounts of protein lysates from each sample were loaded and electrophoresed on a 10% (w/v) Tris–glycine SDS polyacrylamide gel. Following electrophoresis, the proteins in the gel were transferred onto a polyvinylidene fluoride (PVDF) membrane using a transfer buffer. The membrane was subsequently blocked with PVDF Blocking Reagent for Can Get Signal® (TOYOBO CO., LTD., Osaka, Japan) at room temperature for 1 h. The membranes were washed five times with Tris-buffered saline containing 0.05% (v/v) Tween 20 (TBST). Then, the membranes were incubated with an anti-phospho inhibitor kappa B alpha (IκBα) (Ser32/36) antibody (SC-101713, Santa Cruz Biotechnology, Inc., Dallas, TX, USA) or an anti-IκBα antibody (SC-371, Santa Cruz Biotechnology, Inc.) diluted 1000-fold with Can Get Signal® solution 1 at 4 °C overnight. After washing with TBST, the membranes were incubated with an anti-rabbit IgG-horseradish peroxidase (Cell Signaling Technology, Inc., Danvers, MA, USA) diluted 2500-fold with Can Get Signal® Solution 2 at room temperature for 1 h. Finally, after the washing with TBST again, the bands were visualized with LAS-3000 (Fujifilm Corporation, Tokyo, Japan) using an Amersham ECL Select Western Blotting Detection Reagent (GE Healthcare, Buckingham, UK).