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Modification of energy balance induced by the food contaminant T-2 toxin: A multimodal gut-to-brain connection

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ABSTRACT

T-2 toxin is one of the most toxic *Fusarium*-derived trichothecenes found on cereals and constitutes a widespread contaminant of agricultural commodities as well as commercial foods. Low doses toxicity is characterized by reduced weight gain. To date, the mechanisms by which this mycotoxin profoundly modifies feeding behavior remain poorly understood and more broadly the effects of T-2 toxin on the central nervous system (CNS) have received limited attention. Through an extensive characterization of sickness-like behavior induced by T-2 toxin, we showed that its *per os* (*p.o.*) administration affects not only feeding behavior but also energy expenditure, glycaemia, body temperature and locomotor activity. Using c-Fos expression mapping, we identified the neuronal structures activated in response to T-2 toxin and observed that the pattern of neuronal populations activated by this toxin resembled that induced by inflammatory signals. Interestingly, part of neuronal pathways activated by the toxin were NUCB-2/nesfatin-1 expressing neurons. Unexpectedly, while T-2 toxin induced a strong peripheral inflammation, the brain exhibited limited inflammatory response at a time point when anorexia was ongoing. Unilateral vagotomy partly reduced T-2 toxin-induced brainstem neuronal activation. On the other hand, intracerebroventricular (icv) T-2 toxin injection resulted in a rapid (<1 h) reduction in food intake. Thus, we hypothesized that T-2 toxin could signal to the brain through neuronal and/or humoral pathways. The present work provides the first demonstration that T-2 toxin modifies feeding behavior by interfering with central neuronal networks devoted to central energy balance. Our results, with a particular attention to peripheral inflammation, strongly suggest that inflammatory mediators partake in the T-2 toxin-induced anorexia and other symptoms. In view of the broad human and breeding animal exposure to T-2 toxin, this new mechanism may lead to reconsider the impact of the consumption of this toxin on human health.

1. Introduction

Fusarium fungi are common parasites that largely infect cereals including wheat, maize, barley, rye, oats or rice, with cereals contaminations reported in all parts of the world and especially in temperate regions of Europe, North America and Asia. These fungi produce various metabolites among which some of them called trichothecenes have adverse effect in humans and animals. Moreover, given their stability during processing and/or cooking, trichothecenes could be present in animal and human food commodities (Schothorst and van Egmond, 2004; Streit et al., 2012; Van Der Fels-Klerx et al., 2012). Trichothecenes can be divided into two groups namely type A and B. While toxins of type B, including Deoxynivalenol (DON) or nivalenol, are the most common trichothecenes found in food commodities, type A toxins such as T-2 toxin are considered as the most toxic (Joint FAO/WHO expert Committee on Food Additives (JECFA) 2001). T-2 toxin can be detected in agricultural commodities as well as some commercial foods (Van der Fels-Klerx, 2012). For instance, an evaluation of T-2 toxin occurrence in human food commodities performed in Europe reported that 20% out of a total of 3490 cereals-derived samples analyzed were positive (Schothorst and van Egmond, 2004). This leads to a daily exposure that largely exceeds the Tolerable Daily Intakes (TDI) for a large number of European citizens and especially for young children (Schothorst and van Egmond, 2004; Thuvander et al., 2001). T-2 toxin is known to cause extensive pathology in animals and humans (Hsu et al., 1972; Ueno et al.,

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1972; Wyatt et al., 1972; Joffe, 1983). In animals, T-2 toxin induces behavioral and physiological changes such as food refusal, vomiting, lethargy, ataxia, hemorrhages, sepsis and cardiopulmonary failure. As mentioned above, T-2 toxin poisoning was shown to alter food consumption in different models including bird (Chi et al., 1977; Burditt et al., 1983), swine (Rafai et al., 1995) and rodents (Hayes and Schiefer, 1982). In rodents, the acute toxic effects of dietary T-2 toxin were compared in rats and mice by Hayes and Schiefer (1982). T-2 toxin added to the diet at levels of 10 or 20 ppm for 2 or 4 weeks induced a dose-related depression in food consumption and weight gain in all animals. A result confirmed in rats by Carson and Smith (1983) who reported that rats fed during 2 weeks with diets supplemented with T-2 toxin at 3 μg/g exhibited a depressed feed consumption and a body weight loss. To date, the mechanisms by which this mycotoxin profoundly modifies the feeding behavior remain poorly understood, and more broadly the effects of T-2 toxin on the central nervous system (CNS) have received limited attention. In the CNS, the regulation of appetite relies on complex neurocircuitry (Morton et al., 2006). Discrete neuronal pathways within specific brain areas, mainly the hypothalamus and the brainstem, are clearly involved in this control of feeding behavior. Peripheral information linked to fat deposit and nutriment availability are implicated as endogenous signaling molecules in the control of energy expenditure, and initiation and termination of a meal. During the eighties and nineties, different groups tried to characterize the neurochemical modifications observed in the brain of T-2 toxin poisoned animals and to explain behavioral changes. Alterations of whole brain monoamines and/or serotonimergic concentrations were reported by several authors in different animal models (Chi et al., 1981; MacDonald et al., 1988; Boyd et al., 1988; Weekley et al., 1989; Wang et al., 1998a). However, these results are sometimes conflicting and clearly appear insufficient to explain the decreased food intake behavior observed in response to T-2 toxin. Furthermore, in the absence of any food intake monitoring during the pioneer studies, no correlation could be drawn between the time course of T-2 toxin-evoked anorexia and brain neurochemistry alterations.

In the present study, combining a multidisciplinary approach on a mice model, we sought to determine the gut-to-brain connections and the central mechanisms underlying the physiological and behavioral alterations observed during T-2 toxin intoxication.

2. Materials and methods

2.1. Animal housing

Experiments were performed on adult male C57Bl6 mice 20–25 g in body weight (Charles River, France). All animals were individually housed in a facility at controlled temperature on a 12/12-h light/dark cycle (lights on at 7 am) with standard powder diet (AO4 P2.5, SAFE UAR, France) and water available *ad libitum*. Individual cages were designed in order to limit spillage (Pecchi et al., 2008). Mice had free access to standard powder diet *via* two holes made at the bottom of the cage. For habituation, animals were housed in these cages at least 10 days before experiments. All experiments were performed at 22 °C. Experiments carried out in this study were performed in strict accordance with European Economic Community guidelines (86/609/EEC) and the local committees' recommendations (C-13-055-6, Aix-Marseilles University) for the care and use of laboratory animals.

2.2. Per os administration of T-2 toxin

At the beginning of the dark phase, mice were orally administered 0.5–5 mg/kg body weight (bw) T-2 toxin (T4887, Sigma Aldrich, France) *via* gavage, using a 22 gauge intubation needle (Popper and Sons). 5 mg of T-2 toxin was dissolved in 100 μl dimethyl sulfoxide (DMSO) and final dilution was made in distilled water. The final DMSO concentration administered to animals was comprised between 0.2% and 2%. Mice received 5 μl/g (bw) of each solution. The same volume of distilled water containing the corresponding DMSO percentage was given to control animals. Prior to T-2 toxin treatment, mice received the same volume of distilled water with the same oral administration procedure for a habituation period of seven consecutive days.

2.3. Surgery and intracerebroventricular injection of T-2 toxin

Cannula implantation: Animals were anaesthetized by an intraperitoneal (ip) injection of ketamine (100 mg/kg; Imalgen 1000, Merial, France) and xylazine (16 mg/kg; Rompun, Bayer Santé, France), and placed in a digital stereotaxic apparatus (Model 502600, WPI) coupled to the neurostar software (Neurostar GmbH). A 26-gauge stainless steel cannula was implanted into the lateral ventricle at the following coordinates: 0.3 mm posterior to bregma, 1.1 mm lateral to the midline, and 2.6 mm ventral to the skull surface. The cannula was secured to the skull with dental cement and sealed with removable obturators. The animals were sutured, placed in individual cages and allowed to recover for 7 days. During this resting period, animals were injected with physiological saline every other day for habituation. One week post-surgery, mice were administered 10 μl (2.5 μl/min) of either physiological saline or T-2 toxin (0.5 μg/μl) solution at the beginning of the dark phase.

2.4. Vagotomy surgery

For unilateral cervical vagotomies (UCV), animals were anaesthetized with an ip injection of ketamine (100 mg/kg; Imalgen 1000, Merial,) and xylazine (6 mg/kg; Rompun, Bayer). Nerve section was performed as previously described (Pecchi et al., 2007). The left vagus nerve, which transfers the majority of gastrointestinal signals to the CNS, was sectioned. In the contralateral side of the section, the carotid trunk was exposed but the vagus nerve was not injured. Animals were allowed to recover from surgery for 10 days before T-2 toxin administration.

2.5. Telemetry measurements

Body temperature and locomotor activity were recorded using TA10TA-F20 telemetry probes (Data Sciences International). Mice were anaesthetized as previously described. A telemetry probe was implanted ip in each animal. After surgery, mice were housed individually, maintained at a constant temperature of 22 °C and placed on a receiver RPC-1 (Data Sciences International). Telemetry radio signals emitted by the implanted transmitter were relayed to the data acquisition system via a consolidation matrix, converted into temperature and locomotor activity data using Dataquest ART 4.2 data acquisition software, and recorded every 5 min. Mice were then administered water by oral gavage daily just before lights off for habituation. One week after surgery, mice received water containing DMSO as a control and one day later they received T-2 toxin (0.5, 2 and 5 mg/kg) by oral gavage.

2.6. Food intake measurements

Powdered food consumption: Just before lights off, mice received intra-esophageal administration of T-2 toxin or vehicle. Immediately after treatment, a fresh supply of pre-weighed food was given. The measurement of powdered food intake was the same as in previous studies (Girardet et al., 2011a,b). Food intake was