Central Inflammation and Sickness-Like Behavior Induced by the Food Contaminant Deoxynivalenol: A PGE2-Independent Mechanism

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Deoxynivalenol (DON), one of the most abundant trichothecenes found on cereals, has been implicated in mycotoxicoses in both humans and farm animals. Low-dose toxicity is characterized by reduced weight gain, diminished nutritional efficiency, and immunologic effects. The levels and patterns of human food commodity contamination justify that DON consumption constitutes a public health issue. DON stability during processing and cooking explains its large presence in human food. We characterized here DON intoxication by showing that the toxin concomitantly affects feeding behavior, body temperature, and locomotor activity after both per os and central administration. Using c-Fos expression mapping, we identified the neuronal structures activated in response to DON and observed that the pattern of neuronal populations activated by the toxin resembled those induced by inflammatory signals. By real-time PCR, we report the first evidences for a DON-induced central inflammation, attested by the strong upregulation of interleukin-1β, interleukin-6, tumor necrosis factor-α, cyclooxygenase-2, and microsomal prostaglandin synthase-1 (mPGES-1) messenger RNA. However, silencing prostaglandins E2 signaling pathways using mPGES-1 knockout mice, which are resistant to cytokine-induced sickness behavior, did not modify the responses to the toxin. These results reveal that, despite strong similarities, behavioral changes observed after DON intoxication differ from classical sickness behavior evoked by inflammatory cytokines.

Key Words: mycotoxin; cytokines; brain; mPGES-1; c-Fos.

Fusarium fungi are common pathogen growing on cereals cultivated in Europe, America, and Asia. The extent of cereal contamination is strongly associated with rainfall and moisture and with grain storage conditions. These fungi produce various mycotoxins, which contaminate animals and human food commodities. The trichothecene deoxynivalenol (DON), also commonly called vomitoxin, is one of the most abundant mycotoxin found on contaminated cereals, and its stability during processing and cooking explains its widespread presence in human food (Lombaert et al., 2003; Schothorst and van Egmond, 2004; Turner et al., 2008, 2010a). Moreover, farm workers who handle grain or silage may be at additional risk exposure to DON (Turner et al., 2010b). DON has been shown to be implicated in acute and chronic illnesses in both humans and farm animals. Whereas high-dose toxicity of DON is characterized by a set of symptoms including diarrhea, vomiting, leukocytosis, hemorrhage, circulatory shock, and death, low-dose toxicity is characterized by anorexia, reduced weight gain, diminished nutritional efficiency, neuroendocrine changes, and immunologic effects (for review, see Pestka, 2010). In view of this widespread human exposure to DON, studies improving our knowledge of DON toxicity are essential and should be conducted. DON was reported to have modulatory effects on the immune system. Low doses of DON upregulate the expression of cytokines and chemokines, whereas high doses exert immune suppression action via the induction of leukocyte apoptosis. Oral exposure to DON at 5 mg/kg was shown to increase, shortly after treatment (2 h), the expression of proinflammatory cytokines such as interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α), and interleukin-6 (IL-6) in the spleen, liver, lung, and kidney (Amuzie et al., 2008; Pestka and Amuzie, 2008). This stimulatory effect of low DON doses could be explained by both an increased cytokine gene transcription and an enhancement of their messenger RNA (mRNA) stability. It is now well admitted that the proinflammatory cytokines produced during infection or inflammation allow communication between the immune and central nervous systems (CNS). In response to cytokine actions, the CNS coordinates a set of nonspecific symptoms referred to as sickness behavior, which notably includes anorexia, modulation of body core temperature, and reduced activity. During infection, cells of
the innate immune system located at the periphery or in the CNS recognize specific components of microorganisms, which in turn induce the synthesis and release of inflammatory cytokines such as IL-1β, TNF-α, and IL-6. These soluble mediators coordinate the local and systemic inflammatory responses and act on the brain to induce the central symptoms specific to sickness. Among the inflammatory cytokines, IL-1β seems to be the most potent in inducing sickness behavior because its peripheral or central injection reproduces the set of nonspecific symptoms of inflammation including decreased motor activity, social withdrawal, anorexia, and fever (Kelley et al., 2003). The injection of TNF-α or IL-6 also induces anorexia, fever, decreased activity, and social withdrawal. Although the direct action of blood-borne cytokines on brain structures has been proposed to mediate sickness behavior, the de novo cytokine synthesis in discrete areas of the brain in response to peripheral inflammation has also been largely illustrated. Moreover, it is well recognized that a complex neuronal circuitry activated in response to inflammation coordinates the behavioral responses observed during infection and inflammation.

The concomitant induction of anorexia and upregulation of proinflammatory cytokines by DON emphasize the strong similarities between sickness behavior resulting from inflammatory challenge and DON intoxication. Some authors have indeed proposed that, given the well-characterized anorexigenic action of these inflammatory molecules, they could partake in the toxin-induced anorexia (Pestka et al., 2004). However, although the peripheral action of DON was extensively studied (Pestka, 2010), data illustrating its effects on CNS are significantly less abundant. Despite the well-described modulation of feeding behavior induced by DON consumption, the data aiming to characterize the symptoms associated with this loss of appetite and the central mechanisms by which this toxin exerts its actions remain largely unknown.

In the present study, combining a multidisciplinary approach and a mice model of DON intoxication, we characterized the symptoms associated with anorexia during acute oral and central administrations of DON and identified the inflammation biomarkers whose central expression is upregulated by the toxin. Finally, using a mice genetic model characterized by an impaired Prostaglandins E2 (PGE2) synthesis, we demonstrated that DON-induced sickness-like behavior is independent of PGE2 action.

**MATERIALS AND METHODS**

**Animal Housing**

Experiments were performed either on adult male wild-type (WT) (20–25 g) or microsomal prostaglandin synthase-1 (mPGES-1) −/− (18–25 g) DBA/ILac J mice. mPGES-1 −/− mice exhibit a deletion of the Pges gene, which encodes mPGES-1 (Trebino et al., 2003). mPGES-1 −/− colony was provided by Prof. F. Berenbaum (Pierre and Marie Curie University, Paris) and has been characterized previously (Pecchi et al., 2006). Historically, DBA/ILac J mice strain was used to develop arthritis models induced by immunization with type II collagen. This genetic background was next chosen for mPGES-1 invalidation and subsequent characterization of mPGES-1 −/− phenotype in response to various inflammatory conditions. In the present study, both WT and knockout (KO) mice were obtained from heterozygous mPGES-1 mice mating. All animals were individually housed in a pathogen-free facility at controlled temperature on a 12/12-h light/dark cycle (lights on at 7 A.M.) with standard powder diet (AO4 P2.5, SAFE UAR) 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 for least 10 days before experiments. All experiments were performed at 21°C except when specified. Experiments carried out in this study were performed in strict accordance with European Economic Community guidelines (86/609/EEC) and the local committee’s recommendations (C-13-055-6, Aix-Marseille University) for the care and use of laboratory animals.

**Per os Administration of DON**

One hour prior to the beginning of the dark phase, mice were orally administered 6.25, 12.5, and 25 mg/kg body weight (bw) DON (D-0156, Sigma Chemical Co., 98% purity) and its purity verified by high-performance liquid chromatography with diode-array detection set at 220 nm. Mice received an injection volume of 100 μl/g bw of DON dissolved in distilled water via gavage, using a 22-gauge intubation needle (Popper and Sons). Prior to DON treatment, mice received the same volume of distilled water using the similar oral administration procedure for a seven consecutive days of habituation period.

**Surgery and Intracerebroventricular Injection of DON**

**Cannula implantation.** Animals were anesthetized by an ip injection of ketamine (100 mg/kg; Imalgem 1000, Merial) and xylazine (6 mg/kg; Rompun, Bayer) and placed in a digital stereotaxic apparatus (Model 502600, WPI) coupled to the Stereodrive 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 after surgery, mice were administered either 10 μl (2 μl/min) physiological saline or DON solution (20 μg per mouse) at the beginning of the dark phase. This dose of DON was chosen because it is known to not cause anorexia when administered at the periphery. The correct cannula positioning was checked for each animal at the end of experiment by cresyl violet staining of brain sections. In some cases, a TA10TA-F20 telemetry probe (Data Sciences International) was implanted ip as soon as the cannula implantation procedure was finished.

**Food Intake Measurements**

**Powdered food consumption.** Experiments were performed on animals allowed to eat throughout the day. One hour before lights off, mice received either intraesophageal or intracerebroventricular (ICV) administration of DON or vehicle. Immediately after treatment, a fresh supply of preweighed food was given. The measurement of powdered food intake was the same as in previous studies (Pecchi et al., 2008). Food intake was calculated as the difference between the preweighed and the remaining powder measured with a precision balance (0.01 g; Denver Instrument from Bioblock).

**Telemetry Measurements**

Body temperature and locomotor activity were recorded using TA10TA-F20 telemetry probes (Data Sciences International). Mice were anesthetized as previously described. A telemetry probe was implanted ip in each animal. After surgery, mice were housed individually, maintained at a constant temperature of 21°C or 31°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 at 6 pm for habitation. One week after surgery, mice received water as a control and 3 days later, they received DON, both by oral gavage.

Quantitative RT-PCR Analysis

Animals used for real-time polymerase chain reaction (RT-PCR) analysis were not refed after DON administration and were sacrificed 3 h after treatment. mRNA expression within the brainstem and the hypothalamus was quantified as previously described (Pecchi et al., 2008). Briefly, total RNA was extracted from frozen hypothalamus and brain stem using RNeasy Mini Kit and RNeasy Micro Kit (Qiagen), respectively. Reverse transcription of the RNA was performed using random primers and M-MLV reverse transcriptase (Promega). Gene expression analysis by real-time PCR was performed using the Applied Biosystems 7500 Fast Real-Time PCR System (Applied Biosystems). The equivalent of 6.25 ng initial RNA was subjected to PCR amplification in a 10 μl final volume using 2.4 μM specific primers and SYBR Green PCR Master Mix (Applied Biosystems). Product formation (primers in Table 1) was detected at 60°C in the fluorescein isothiocyanate channel. The generation of specific PCR products was confirmed by melting-curve analysis. For each PCR, complementary DNAs (cDNAs) were run in duplicate in parallel with serial dilutions of a cDNA mixture tested for each primer pair to generate a standard linear curve, which was used to estimate relative quantities of each gene of interest and of β-actin (internal reference gene) mRNA.

Immunohistochemistry Procedures

As mentioned for PCR analysis, per os–treated animals used for the immunostaining procedure were sacrificed 3 h after treatment without any access to food. Animal perfusions were achieved with 10 ml of 0.1M PBS followed by 50 ml of 4% paraformaldehyde (PFA) in 0.1M PBS. Brains were postfixied for 1 h in 4% PFA at room temperature, rinsed in PBS, and then cryoynpped for 24–48 h in 30% sucrose at 4°C. After freezing of the brains in isopentane (−40°C), coronal sections (40 μm thick) were cut on a cryostat (Leica CM3050, France) and collected serially in PBS (0.1M, pH 7.4) from caudal brain stem (Bregma −8.24 mm) to forebrain (Bregma +0.75 mm). c-Fos immunohistochemistry was performed on free-floating sections using an anti-c-Fos rabbit antiserum synthesized against amino acids 4–17 of human c-Fos immunohistochemistry was performed on free-floating sections using an anti-c-Fos rabbit antiserum synthesized against amino acids 4–17 of human c-Fos. The antibody diluted in the same solution. A biotinylated goat anti-rabbit IgG was visualized using a nickel-enhanced diaminobenzidine as the chromogen. Activity. After blocking with PBS containing 3% normal goat serum and 0.3% Triton X-100, sections were incubated for 48 h at 4°C from c-Fos–stained elements were identified by setting a threshold value. Counts were manually corrected to avoid overlapping cell nuclei and count of positive objects whose surface area did not exceed 3 μm².

Statistical Analysis

Data are represented as mean ± SEM. Comparisons between data from vehicle- and DON-treated mice were performed using unpaired two-tailed Student’s t-test. One-way ANOVA were performed for comparison between data from mice treated with different doses of DON. For experiments on KO mice and at 31°C, data were compared with respective controls (WT and 21°C) with two-way ANOVA, with treatment (DON vs. vehicle) and, respectively, either genotype (WT vs. KO) or ambient temperature (21°C vs. 31°C) as factors. Tukey’s Honestly Significant Difference test was used for post hoc analysis. p Values less than 0.05 were considered significant.

RESULTS

Effect of Acute per os DON Administration on Food Intake, Locomotor Activity, and Body Core Temperature

A single oral administration of DON resulted in a dose-dependent decrease in daily food intake with a notably long-lasting effect for the highest doses (Fig. 1A). Although food consumption returned to basal level after 24 h with the 6.25 mg/kg dose, eating was affected up to 48 h after treatment with 12.5 and 25 mg/kg. Cumulative food intake measured over a period of 24 h, i.e., 3, 6, 12, 18, and 24 h after treatment, revealed that DON profoundly affected the nighttime food intake (Fig. 1B). In parallel to the alteration of feeding behavior, DON also caused a dose-dependent reduction of nightly locomotor activity (Fig. 2). The rhythm of locomotor activity was durably affected by the DON treatment especially with the 12.5 and 25 mg/kg DON doses with normal activity amplitude reappearing during the night of the third day (Fig. 2A). Because changes in body temperature are features associated with anorexia and reduced locomotor activity during sickness behavior consecutive to infection, we next examined the impact of DON on body core temperature. At an ambient temperature of 21°C, per os DON administration at the end of the light phase resulted in a decrease in body temperature during the first 2 h after DON challenge (Fig. 3B). This effect was more pronounced with the highest doses, i.e., 12.5 and 25 mg/kg (Figs. 3A and 3B). With these doses, the DON-induced decrease in temperature reached up to 2.5°C ± 0.4°C in the first 2 h followed by a long-lasting hypothermia, which affected daily temperature rhythm amplitudes during the following 72 h (Fig. 3A). To evaluate the dependence of DON-induced hypothermia on ambient temperature, additional experiments

TABLE I

References of Primers Used (Qiagen) for SYBR Green Assays

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Microscopy, Image Analysis, and Cell Count

c-Fos immunostaining was analyzed by counting the positive nuclei on four adjacent sections. c-Fos–positive nuclei counting was performed on photomicrographs acquired using a 10-fold lens with a DXM 1200 Camera (Nikon) coupled to ACT-1 software. The microscope was set at a specific illumination level, as was the camera exposure time. c-Fos–positive nuclei were then counted on these pictures by computer-assisted morphometry using the NIH image J software as previously described (Dallaporta et al., 2007). Briefly, images were normalized by subtracting the background determined for each structure studied, and c-Fos–stained elements were identified by setting a threshold value. Counts were manually corrected to avoid overlapping cell nuclei and count of positive objects whose surface area did not exceed 3 μm².
were performed with an ambient temperature of 31°C. Under these conditions, DON still induced a decrease in body temperature (Fig. 3C).

**Effect of ICV DON Injection on Sickness-Like Behavior**

To determine whether a central action of DON could result in the same symptoms as those observed after per os administration, we next performed ICV injections (lateral ventricles) of the toxin on chronically cannulated mice. As observed after per os administration, central DON injections (20 µg per mouse) reduced food intake measured during the dark phase (Fig. 4A). Cumulative food intake measured over a 12-h period revealed that DON profoundly affected food intake as soon as the first hour and the effect was long lasting during the following 12 h after injection. Food intake was indeed reduced by more than 70% six and 9 h after injection. The anorexigenic effect of centrally injected DON was still observable 12 h after injection. When administered peripherally, the same DON dose, which is about 10- to 30-fold lower than the per os administered DON doses (equivalent to 150–600 µg per mouse), failed to reduce food intake. It should be noticed that ICV DON injection resulted in a decrease in body core temperature with a maximum of −3. 5 ± 0.9°C reached 7 h after injection (Fig. 4B). Regarding locomotor activity, ICV DON injection resulted in a biphasic modulation of this parameter. Whereas locomotor activity appeared to be increased during the 3 h following DON injection, a strong decrease of this behavior was observed in the second part of the dark phase (Fig. 4C).

**Central Expression of Inflammation Biomarkers during per os DON Intoxication**

Because DON was previously shown to increase proinflammatory cytokine gene expression in peripheral tissues, we next investigated whether DON administration (12.5 mg/kg) could result in an upregulation of these genes in the brain. Real-time PCR was performed on transcripts isolated from two central structures: the dorsal vagal complex (DVC), which constitutes a gateway for immune information from the periphery to the brain, and the hypothalamus, which coordinates feeding behavior. Three hours after DON administration, IL-1β, TNF-α, and IL-6 mRNAs were strongly upregulated both in the hypothalamus and the DVC (Fig. 5). PGE2 are key effectors of sickness behavior observed during inflammatory states. Analysis of transcripts revealed that DON induced a strong upregulation of the PGE2-synthesizing enzymes cyclooxygenase-2 (COX-2) and mPGES-1 mRNA expression, 3 h after the injection, in both structures studied (Fig. 5).

**DON-Induced Intoxication in mPGES-1 KO Mice**

Based on these observations, we next evaluated the impact of DON treatment on mPGES-1–lacking mice (mPGES-1−/−). A single oral administration of 12.5 mg/kg DON resulted in a similar reduction of food intake up to 48 h after treatment in both WT and mPGES-1−/− (Fig. 6A). The quantification of food consumption 3, 6, 12, 18, and 24 h after DON administration revealed that DON affected the dark phase food consumption similarly in WT and mPGES-1−/− mice (Fig. 6B). Locomotor activity and body core temperature analysis did not reveal any difference in response to per os DON administration between WT and mPGES-1−/− treated mice (Fig. 7). Central structures activated in response to per os DON administration were next identified using the immune detection of the early gene product c-Fos. A very low basal level of c-Fos–positive nuclei was observed in the brain stem, pons, and forebrain of water-treated mice whatever the genotype considered (Table 2). DON-treated WT mice exhibited a strong rise in the number of c-Fos–positive nuclei within the nucleus tractus solitarius (NTS) and a moderate increase within the area postrema (AP, Fig. 8, Table 2) and ventrolateral medulla. Noticeably, the serotonergic raphe formation did not exhibit any DON-induced c-Fos expression whatever the rostrocaudal level considered (Fig. 8, A). Daily food intake (percentage of initial food intake) measured from 0 to 72 after oral gavage of either water (vehicle) or DON (6.25, 12.5, and 25 mg/kg) in adult mice. (B) Cumulative food intake (g) measured over the first 24-h period of mice having received an oral gavage of either water or DON (6.25, 12.5, and 25 mg/kg). In (A), ‘‘#’’ and ‘‘†’’ represent p < 0.05, significantly different from vehicle-treated mice, respectively, for 6.25, 12.5, and 25 mg/kg DON-treated mice. In (B), *p < 0.05 and **p < 0.01, significantly different from vehicle-treated mice.
WT animals challenged with DON also displayed a strong rise in c-Fos immunoreactivity in the lateral part of the parabrachial nucleus and in the locus coeruleus (LC) and a moderate-to-strong c-Fos staining within forebrain structures such as the paraventricular hypothalamus nucleus (PVN), arcuate nucleus, median eminence (ME), bed nucleus of the stria terminalis, and central nucleus of the amygdala (CeA) (Fig. 8, Table 2). c-Fos analysis failed to reveal any large differences in the pattern and intensity of cellular activation between DON-treated WT and mPGES-1/C0/C0 mice (Fig. 8, Table 2). Only the CeA of mPGES-1/C0/C0 mice exhibited a higher number of c-Fos–positive nucleus in response to DON when compared with WT counterparts.

**DISCUSSION**

**DON-Induced “Sickness Behavior” Symptoms in Mice**

The present study was performed using mice as a model. Although less sensitive to DON than pig, mice can nevertheless be considered as a good model for in vivo DON toxicity studies (Arnold et al., 1986; Flannery et al., 2011; Rotter et al., 1992). We report here that per os DON intoxication modified food intake and affected body core temperature and spontaneous locomotor activity. The anorexic action of DON measured here is in accordance with previous works (for review, Pestka, 2010). Recently, Flannery et al. (2011) reported the characterization of DON-induced anorexia using mouse as a model. These authors reported that the reduction of food intake induced by DON exposure is followed by an orexigenic effect observed 14 h after the treatment. Such a rebound in food intake was not observed in the present study. The dissimilar protocols used in these works may explain this difference. Whereas Flannery et al. (2011) proceed to a fasting during the light cycle before DON exposure, the present study was performed on animals with free access to food throughout the day. We showed that concomitantly to anorexia, DON induced a fall in locomotor activity and body temperature shortly after administration. The hypothermia observed here in rodents was evocative of the decreased skin temperature observed in young pigs fed low dietary concentrations of DON (Rotter et al., 1994) and of the hypothermia monitored after an exposure to the mycotoxin T-2 (Taylor et al., 1991) or to cisplatin (Vera et al., 2006). We bring here evidence showing that centrally
injected DON, at doses ineffective at the periphery, was able to reproduce behavioral features observed after per os intoxication. Centrally administered DON induced hypothermia and modulation of locomotor activity in addition to a rapid (< 1 h) reduction in food intake. These results suggested that a part of the orally administered toxin could reach the brain through circumventricular organs. The DON-induced c-Fos staining observed in circumventricular organs and surrounding structures (AP, NTS, ME, and Arc) supported this hypothesis. Moreover, DON was shown to be rapidly distributed in various organs including the brain within a short time after peripheral exposure (Pestka et al., 2008). The symptoms observed in response to DON were evocative of nonspecific symptoms induced by inflammatory challenges. Acute infections and other immune challenges trigger in the host a generalized defense response termed “acute phase reaction,” which

FIG. 3. Monitoring of body core temperature during per os DON intoxication. (A) Recording of body core temperature over 96 h of mice treated with either vehicle (water) or DON (12.5 mg/kg). (B) Effect of increasing DON doses (6.25, 12.5, and 25 mg/kg) on nightly body core temperature. (C) Effects of DON (12.5 mg/kg) administration performed at different ambient temperature (21°C vs. 31°C) on nighttime body core temperature. In (A), *p < 0.05, significantly different from vehicle-treated mice.
comprises immune, physiological, and behavioral changes. Sick individuals develop a set of nonspecific symptoms, which includes emesis, anorexia, fever, lethargy, and reduction of social interactions, referred to as sickness behavior (for review, see Hart, 1988). It is well established that a complex neuronal circuitry activated in response to inflammation coordinates sickness behavior. Using c-Fos staining, which remains a useful approach to identify activated neuronal groups (Dragunow and Faull, 1989), we revealed the activation of key autonomic areas, hypothalamic nuclei, and parts of the amygdala. This analysis was performed 3 h after peripheral DON administration. At this time point, structures found activated could be considered instrumental in DON-induced responses as anorexia and associated symptoms were ongoing. The c-Fos pattern observed is consistent with a coordinated autonomic, endocrine, and behavioral response to DON. c-Fos

**FIG. 4.** Effect of ICV DON injection. (A) Food intake of mice having received an ICV injection of either saline (vehicle) or DON (20 μg per mouse). Effect of ICV DON injection (20 μg per mouse) on body core temperature (B) and locomotor activity (C). *p < 0.05, **p < 0.01, and ***p < 0.001, significantly different from vehicle-treated mice.

**FIG. 5.** Central expression of inflammatory biomarkers during DON-induced intoxication. IL-1β, IL-6, TNFα, COX-2, and mPGES-1 transcript expressions in the hypothalamus and the DVC quantified 3 h after the oral administration of vehicle (water, white bars) or DON (12.5 mg/kg, purple bars). *p < 0.05 and **p < 0.01, significantly different from vehicle-treated mice.
immunoreactivity observed in key brain stem and hypothalamic nuclei such as NTS, Arc, and PVN is consistent with the DON-induced reduction in food intake. Moreover, the observed c-Fos induction within the brain stem and particularly the AP was evocative of nausea-induced anorexia. Using systemic DON administration in rats, Ossenkopp et al. (1994) have indeed reported the induction of conditioned taste aversion, which was mediated by the AP. The strong increase in c-Fos immunoreactivity in specific regions of the hypothalamus including the preoptic area/ anterior hypothalamus (Boulant, 2000), the PVN and supraoptic nucleus, together with the activation of LC and NTS (Takahashi et al., 2001), could explain the decreased body temperature observed in response to DON. As mentioned earlier, the observed responses to DON, i.e., nausea/emesis, anorexia, modulation of body temperature, and reduced locomotor activity, are also features of sickness behavior that follows an infection. In accordance, the pattern of c-Fos distribution throughout the brain observed after per os DON administration was also consistent with the findings of studies performed to identify immunosensitive neurocircuity involved in the coordinated responses to inflammatory challenges (Lacroix and Rivest, 1997). However, unlike in the case of a response to inflammation, we did not observe any significant elevation of c-Fos immunoreactivity in the ventromedial preoptic area, a cell group adjacent to the organum vasculosum of the lamina terminalis. Finally, c-Fos expression observed in the brain after DON exposure is evocative of previous results showing that in swine, DON intoxication induced changes in extracellular levels of neurotransmitters such as norepinephrine, dopamine, and serotonin in discrete areas of the brain including the hypothalamus and the medulla (Prelusky et al., 1992).
Neuroinflammation Induced by DON Exposure

DON has been reported to modulate the immune system in murine models and to increase cytokine production in both in vitro and in vivo murine models (for review, see Pestka, 2010). However, the data illustrating the possible stimulating effect of DON on cytokine production within the CNS are still lacking. Yet, the development of sickness behavior in response to infection is strongly associated not only with the peripheral production of cytokines but also with their central induction. The role of de novo cytokine synthesis in discrete areas of the brain has also been illustrated by recent works (Chakravarty and Herkenham, 2005; Wisse et al., 2007). Using genetic models exhibiting impairment of lipopolysaccharide (LPS) and IL-1β signaling, these studies have shown that the central production of cytokines is required to obtain a sustained anorexia in response to LPS or IL-1β. Moreover, we have shown that DON administered at the CNS level provoked anorexia, hypothermia, and activity modulation, suggesting that the peripheral action of DON is not required to observe behavioral changes. In accordance, we showed here that per os DON administration induced an upregulation of IL-1β, IL-6, and TNF-α within two central structures, i.e., hypothalamus and DVC. As mentioned earlier for c-Fos study, mRNA analysis was performed relatively shortly after DON

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<td>++</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Paraventricular nucleus</td>
<td>0/+</td>
<td>+++</td>
<td>0/+</td>
<td>+++</td>
</tr>
<tr>
<td>Supraoptic nucleus</td>
<td>+</td>
<td>+++</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>ME</td>
<td>0/+</td>
<td>++</td>
<td>0/+</td>
<td>++</td>
</tr>
<tr>
<td>Dorosmedian nucleus</td>
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<td>0/+</td>
<td>0/+</td>
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</tr>
<tr>
<td>Ventromedian nucleus</td>
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<td>0/+</td>
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</tr>
<tr>
<td>Lateral area</td>
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<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Posterior area</td>
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<tr>
<td>Parasubthalamic nucleus</td>
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<td>0/+</td>
<td>+++</td>
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<tr>
<td>Arcuate nucleus</td>
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</tr>
<tr>
<td>Brain stem</td>
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<tr>
<td>Dorsal raphe nucleus</td>
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<td>0/+</td>
<td>0/+</td>
</tr>
<tr>
<td>Laterodorsal tegmental nucleus</td>
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<td>+</td>
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<tr>
<td>Parabrachial nucleus</td>
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<td>LC</td>
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<td>+++</td>
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<tr>
<td>Motor trigeminal nucleus</td>
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<tr>
<td>Nucleus of trapezoid body</td>
<td>++++</td>
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<td>AP</td>
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<td>+/+</td>
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<td>Caudal NTS</td>
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<td>Postreinal NTS</td>
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<td>+++</td>
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<tr>
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<tr>
<td>Raphe obscurus nucleus</td>
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<tr>
<td>Raphe pallidus nucleus</td>
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<tr>
<td>Caudal ventrolateral medulla</td>
<td>+</td>
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<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Rostral ventrolateral medulla</td>
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<td>+++</td>
<td>+</td>
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<tr>
<td>Spinal nucleus trigeminal</td>
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<tr>
<td>Other</td>
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<td>Ependymal cells</td>
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<tr>
<td>Choroid plexus</td>
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<td>0/+</td>
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Note. 0, no stained cell; +, 130 cells per section; ++, 31-60 cells; ++++, 61 and more cells. Red color indicates brain structures where c-Fos signal is significantly different between vehicle- and DON-treated mice.
administration at a time point where symptoms are clearly visible. These results constitute the first demonstration that *per os* DON administration results in central neuroinflammation. Interestingly, both studied structures, i.e., DVC and hypothalamus, are involved in the regulation of food intake and constitute gateways for immune information from the periphery to the brain. Moreover, c-Fos immunostaining performed in the present work has confirmed their strong activation in response to *per os* DON treatment. The induction of proinflammatory cytokines within these key structures may explain the DON-induced symptoms. A similar increased cytokine expression within these structures was also observed during anorexigenic immune challenge (Pecchi *et al.*, 2006). TNF-α or IL-1β may act as endogenous cryogen and have been proposed to participate in hypothermia during inflammation (Leon, 2004). The increased expression of TNF-α or IL-1β mRNA within the hypothalamus might be linked to the reduced body core temperature observed here after DON administration. Altogether, these results strongly suggest that not only peripheral but also central-borne cytokines may partake in the onset

**FIG. 8.** Effects of *per os* DON administration on c-Fos immunoreactivity in WT and mPGES-1 −/− mice. (A) Representative coronal sections illustrating the c-Fos labeling observed within brain stem, pons, and forebrain regions of WT (upper panel) and mPGES-1 −/− (lower panel) mice treated with DON (12.5 mg/kg) and sacrificed 3 h after treatment. (B) Quantification of the number of c-Fos immunoreactive nuclei within brain stem, pons, and forebrain nuclei observed in WT and mPGES-1 −/− mice treated either with vehicle (water, white bars) or with DON (12.5 mg/kg, orange bars). *p < 0.05, **p < 0.01, ***p < 0.001, significantly different from vehicle-treated mice. 3V, third ventricle; ARC, arcuate nucleus; cc, central canal; CeA, central amygdala; LPB, lateral parabrachial; LC, locus coeruleus; NTS, nucleus tractus solitarius; PVN, paraventricular nucleus; SON, supraoptic nucleus; X, dorsal motor nucleus of the vagus; ts, tractus solitarius. Scale bar: 100 μm.
of sickness-like behavior observed in response to DON intoxication. The cellular targets of DON within the brain remain to be identified. Given the well-known action of this toxin on innate immune cells such as monocytes and macrophages (Bae and Pestka, 2008; Bae et al., 2010; Islam et al., 2006), microglial cells that are the resident macrophages in the CNS constitute a likely DON target.

**DON-Induced Behaviors Are Independent to PGE2 Production**

In addition to cytokine induction, we reported the central upregulation of PGs synthesizing pathways, i.e., COX-2 and mPGES-1 mRNA. The induction of COX-2 gene expression observed here is consistent with previous studies showing that DON upregulates COX-2 expression *in vitro* (macrophage) and *in vivo* (Peyer’s patches and spleen; Moon and Pestka, 2002, 2003). On the other hand, our results constitute the first evidences of a stimulatory effect of DON on mPGES-1 mRNA expression. mPGES-1 has been described as a regulated enzyme whose expression is stimulated by inflammatory agents at the periphery and within the CNS. Because COX-2 and mPGES-1 cooperate to synthesize PGE2, an emblematic mediator of inflammation (Pecchi et al., 2009), our results suggest a high level of central PGE2 synthesis during DON challenge. Given the well-known action of PGE2 on the CNS during immune challenge, it can be hypothesized that this centrally produced PGE2 could, at least partially, contribute to the emergence of the DON-induced behavioral symptoms. Indeed, during inflammation and the subsequent production of cytokines, PGE2 modifies the central neuronal activity to elicit the symptoms of the sickness behavior (Pecchi et al., 2009). Moreover, the reduction of PGE2 production by indomethacin has been shown to attenuate some of the symptoms of sickness behavior such as anorexia (Lugarini et al., 2002). Accordingly, we next investigated the potential instrumental role of mPGES-1 and related PGE2 in DON-induced neuronal activation and associated symptoms by using mPGES-1 −/− mice. Mice lacking the mPGES-1 gene have more than 95% reduction in PGE2 production during inflammation (Trebin et al., 2005) and exhibit impaired febrile (Engblom et al., 2003) and anorexic responses (Pecchi et al., 2006) during IL-1β challenge. Moreover, invalidation of mPGES-1 enzyme strongly reduces inflammation-induced c-Fos expression in immnosensitive brain regions (Dallaporta et al., 2007). In the present study, neither cell activation nor behavioral changes observed in response to DON were prevented or modified by mPGES-1 deletion. These results highlight the fact that mPGES-1 and related PGE2 were not crucial for DON-induced symptoms. This result reinforces previous observations showing that interfering with inflammatory signaling does not reduce the susceptibility to DON-induced symptoms and especially anorexia. TNF-α receptors KO and IL-6 KO mice do not exhibit any reduced susceptibility to DON-induced anorexia (Pestka and Zhou, 2000, 2002). Yet, it should be mentioned that TNF-α KO and IL-6 KO studies were performed using DON-contaminated food, which might have reduced food appetite and masked a potential protective effect of TNF-α receptors KO and IL-6 invalidation. COX-2 KO mice were reported to exhibit a decrease in body weight comparable to that of WT after 16 weeks consumption of DON, although food intake was not monitored in this study (Jia and Pestka, 2005). Even though inflammation-related effectors are strongly expressed in key central structures during DON intoxication, the arguments supporting their triggering role in DON-induced anorexia remain limited. Emphasizing this discrepancy, we showed that unlike cytokine-induced hypothermia, which was shown to be dependent of ambient temperature in rodents (Krall et al., 2010), DON intoxication resulted in hypothermia both at 21° and 31°C. Overall, our results shed light on the fact that, despite high similarities, infection- and DON-induced sickness behavior involve partially different signaling pathways. This suggests that anti-inflammatory therapeutics targeting the arachidonic acid pathways should not reap huge benefits against behavioral symptoms (anorexia, anapyrexia, and lethargy) associated with acute DON intoxication.

In summary, the present work provides the first demonstration that DON induces a set of nonspecific symptoms and a concomitant central inflammation. Although these results suggest that the central expression of inflammatory effectors could partake in the onset of symptoms as proposed for sickness behavior induced by infection, anti-inflammatory strategies aiming to reduce PGE2 production failed to prevent DON-induced symptoms. Finally, in view of the widespread human exposure to DON, future studies should evaluate the impact of DON-induced central inflammation on brain signaling, especially in individuals suffering from neurological pathologies that involve a clear inflammatory component.

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**REFERENCES**


