Involvement of central microsomal prostaglandin E synthase-1 in IL-1β-induced anorexia

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Pecchi, E., M. Dallaporta, S. Thirion, C. Salvat, F. Berenbaum, A. Jean, and J.-D. Troadec. Involvement of central microsomal prostaglandin E synthase-1 in IL-1β-induced anorexia. *Physiol Genomics* 25: 485–492, 2006; doi:10.1152/physiolgenomics.00306.2005.—In response to infection or inflammation, individuals develop a set of symptoms referred to as sickness behavior, which includes a decrease in food intake. The characterization of the molecular mechanisms underlying this hypophagia remains critical, because chronic anorexia may represent a significant health risk. Prostaglandins (PGs) constitute an important inflammatory mediator family whose levels increase in the brain during inflammatory states, and their involvement in inflammatory-induced anorexia has been proposed. The microsomal PGE synthase (mPGES)-1 enzyme is involved in the last step of PGE_2 biosynthesis, and its expression is stimulated by proinflammatory agents. The present study attempted to determine whether an upregulation of mPGES-1 gene expression may account for the immune-induced anorexic behavior. We focused our study on mPGES-1 expression in the hypothalamus and dorsal vagal complex, two structures strongly activated during peripheral inflammation and involved in the regulation of food intake. We showed that mPGES-1 gene expression was robustly upregulated in these structures after intraperitoneal and intracerebroventricular injections of anorexigenic doses of IL-1β. This increase was correlated with the onset of anorexia. The concomitant reduction in food intake and central mPGES-1 gene upregulation led us to test the feeding behavior of mice lacking mPGES-1 during inflammation. Interestingly, IL-1β failed to decrease food intake in mPGES-1$^{-/-}$ mice, although these animals developed anorexia in response to a PGE_2 injection. Taken together, our results demonstrate that mPGES-1, which is strongly upregulated during inflammation in central structures involved in feeding control, is essential for immune anorexic behavior and thus may constitute a potential therapeutic target.

inflammation; interleukin-1β; cyclooxygenase; prostaglandin E_2

INFECTION AND INFLAMMATION induce a set of symptoms referred to sickness behavior, which is characterized by physiological, endocrine, and behavioral changes including anorexia (1). This decrease in food intake appears advantageous for the host insofar as it both limits energy consumption devoted to the search for food and reduces the food-derived nutriments available for the growth of microorganisms (5). However, chronic anorexia compromises immune defenses and reduces muscle mass and thus may represent a significant health risk. The

characterization of molecular mechanisms underlying inflammatory anorexia remains of considerable interest with potential therapeutic development.

The control of food intake involves central structures that interact to maintain a stable body weight over a long period of time. Among these structures, the hypothalamus and dorsal vagal complex (DVC), which, respectively, integrate adiposity and satiety signals, play a pivotal role (20). It appears likely from recent literature that the anorexia observed during inflammation or infection results from a modulation of central nervous system mechanisms that control physiological food intake (19). During infection and inflammation, proinflammatory cytokines produced by a variety of cells reach and interact with brain regions that control ingestion to produce anorexia. Peripheral immune signals arrive at the brain via humoral communication through circumventricular organs such as the anteroventral region of the third ventricle and area postrema and via a neural-immune gut-brain pathway involving, principally, sensory vagal afferents (for a review, see Ref. 9). The vagus nerve constitutes an important neuroanatomic linkage between the gut and central nervous system. Accordingly, anorexic doses of peripheral cytokines such as IL-1β activate the primary projection area of vagus nerves, which is located in the DVC. The multiple secondary projections sites of these nerves involved in the processing of gut visceral information and the control of food intake, i.e., the hypothalamus; the central nucleus of the amygdala and bed nucleus of the stria terminalis are also activated (4a). Finally, a modulation in the expression of neurotransmitters and anorexigenic or orexigenic neuropeptides in the hypothalamus has been proposed to contribute to the anorexic behavior induced by inflammatory signals (for a review, see Ref. 9).

Prostaglandins (PGs) are inflammatory mediators whose levels increase in the brain during inflammatory states and that modify central neuronal activity to evoke some symptoms of sickness behavior (16). PGs are short-lived biomolecules that act near to their site of synthesis. Tissue-specific responses depend on which PGs are generated. PGH_2, the end product of cyclooxygenase (COX) enzymes, can be converted into various PGs such as PGD_2, $PGF_{2\alpha}$, PGE_2, prostacyclin, and thromboxane A_2 (25), with each compound having its own specific biological activities. Interestingly, a previous study (11) has reported PGE_2 as the most potent PG in inducing anorexia when centrally administered. Microsomal PGE synthase (mPGES)-1 belongs to the membrane-associated proteins in eicosanoid and glutathione metabolism superfamily and catalyses the final step of PGE_2 synthesis (6). Furthermore, mPGES-1 has been described as an inducible enzyme whose

expression is stimulated by proinflammatory agents in several cells and tissues (2, 15, 22). Taken together, these data led us to test the possible involvement of mPGES-1 in anorexia induced by inflammation. To this end, we investigated the expression of mPGES-1 transcripts during inflammation in the two main structures involved in normal and pathological regulation of food intake, i.e., the hypothalamus and DVC.

MATERIALS AND METHODS

Animals

Experiments were performed on adult male Wistar rats (Janvier) weighing 250–300 g. Additional experiments were performed on male adult mice of the DBA/1lac J strain with a deletion of the *Ptges* gene, which encodes mPGES-1 (Pfizer) (26). mPGES-1$^{-/-}$ mice were obtained from heterozygote matings. Wild-type (mPGES-1$^{+/+}$) and heterozygous (mPGES-1$^{+/-}$) littermates arose from the mPGES-1 heterozygous matings. All animals were housed individually in a pathogen-free facility at a controlled temperature on a 12:12-h light-dark cycle (lights off at 18.00 hours) with food (AO4, SAFE UAR) and water available ad libitum (except when specified).

Intraperitoneal Injection of IL-1β

For habituation, animals (rats and mice) were handled and injected intraperitoneally with physiological saline every day for at least 7 days before experiments.

One hour before lights off, rats were weighed and injected intraperitoneally with either physiological saline or IL-1β (16 μg/kg, Amgen). At different times after the injection (3, 6, and 12 h), subgroups of rats were weighed, anesthetized with halothane, and killed by decapitation. The hypothalamus and DVC were dissected with a binocular microscope in ice-cold artificial cerebrospinal fluid (CSF) using an acrylic matrix (Alto). Samples were stored at −80°C until RNA extraction.

In a similar way, mPGES-1$^{-/-}$, mPGES-1$^{+/-}$, and mPGES$^{+/+}$ mice were fasted for 16 h before being weighed and received an intraperitoneal injection of either physiological saline or IL-1β solution (1 μg/animal). One hour after the injection, mice were refed. Food intake was measured each hour during 5 h after the refeeding, and animals were weighed at the end of the experiment. Subgroups of animals were anesthetized with pentobarbital (1.5 mg/kg) and killed by decapitation 2 h after the injection of physiological saline or IL-1β. The brain was quickly removed and sectioned in a cryostat at −30°C (Leica). The hypothalamus and DVC were dissected and stored at −80°C until RNA extraction.

Intraperitoneal Injection of PGE₂

After a 16-h fasting period, mice received an intraperitoneal injection of either PGE₂ solution (20 μg/animal) or physiological saline containing 0.7% ethanol (PGE₂ vehicle). Thirty minutes after the injection, mice were refed. Food intake was measured over 30 min to 3 h after the refeeding.

Surgery and Intracerebroventricular Injection of IL-1β

Cannula implantation. Animals were anesthetized by an intraperitoneal injection of ketamine (100 mg/kg, Imalgen 1000; Merial, France) and xylazine (16 mg/kg, Rompun; Bayer, Germany) and placed in a stereotaxic apparatus (Kopf instrument). A 25-gauge stainless steel cannula was implanted into the fourth ventricle with the use of the following coordinates: 13.3 mm posterior to the bregma, 0.2 mm lateral to the midline, and 7.5 mm ventral to the skull surface. The cannula was secured to the skull with dental cement and sealed with removable obturators. The animals were immediately sutured, placed in individual cages, and allowed to recover for 7 days. During the recovery, an intracerebroventricular injection of 10 μl physiological saline (2.5 μl/min) was performed every other day for habituation.

Verification of cannula placement and potency. Verification of the coordinates was performed in three animals by injecting 10 μl of China ink through the cannula. After their brains were subjected rapid cryofreezing, 40-μm brain sections were realized and observed under an optic microscope (Nikon Eclipse E600W). The presence of the blue colorant in the walls on the fourth ventricle attested the correct placement of the cannula (see Fig. 4A). Furthermore, the potency of each intracerebroventricular cannula was functionally evaluated by measuring the increase in plasma glucose after an intracerebroventricular injection of 4 mg of 2-deoxyglucose in isotonic saline solution (4). Only rats that responded to 2-deoxyglucose with a large plasma glucose rise (Glucotrend Premiun; Boerhinger, Germany) were used for intracerebroventricular injections.

IL-1β injection in the fourth ventricle. One week postsurgery, rats were fasted for 16 h. At 9.00 hours, rats were administered either 10 μl physiological saline or IL-1β solution (10 ng/rat) and were refed. The food consumed was quantified over 1–6 h after the refeeding. One and three hours after the rats had been refed, subgroups of rats were anesthetized with halothane and killed by decapitation. The hypothalamus and DVC were microdissected as described above and frozen. All samples were stored at −80°C until RNA extraction.

Measurement of Food Intake

Overnight food consumption. One hour before lights off, rats received an intraperitoneal injection of IL-1β or physiological saline. Immediately after the injection, a fresh supply of preweighed food was given. Food intake was calculated at 3, 6, and 12 h after the injection by measuring the difference between the preweighed standard chow available and the weight of chow and spilled crumbs at each time.

Fast-refed experiments. Animals (rats and mice) were fasted for 16 h before being injected. Food was removed 1 h before lights off,

Table 1. *Sequences of primers and hybridization temperatures used for RT-PCR*

Gene	Hybridization Temperature, °C	Forward Primer	Reverse Primer
β-Actin (rat, mouse)	60	5′-TTGCTGATCCACATCTGCTG-3′	5′-GACAGGATGCAGAAGGAGAT-3′
COX-1 (rat, mouse)	72	5′-CATGGATCCGGATTGGTGGGGGTAG-3′	5′-ATCTCGAGGGGCAGGTCTTGGTGTTG-3′
COX-2 (rat)	72	5′-CTGTATCCCGCCCTGCTGGTG-3′	5′-ACTTGCGTTGATGGTGGCTGTCTT-3′
COX-2 (mouse)	55	5′-CTCCTTTTCAACCAGCAGTTCC-3′	5′-TCTGCAGCCATTTCCTTCTCTC-3′
cPGES (rat)	52	5′-ACCATGCAGCCTGCTTCTGC-3′	5′-CATGACTGGCCGGATTCTCTC-3′
cPGES (mouse)	64	5′-ATGCAGCCTGCTTCTGCA-3′	5′-TTACTCCAGATCTGGCAT-3′
mPGES-1 (rat, mouse)	60	5′-CTGCTGGTCATCAAGATGTACG-3′	5′-CCCAGGTAGGCCACGGTGTGT-3′
mPGES-2 (rat)	46	5′-CTATCAGGTGGTAGAGGTGAA-3′	5′-CGGACAATATAGTCGAAGGAA-3′
mPGES-2 (mouse)	53	5′-CTATCAGGTGGAGGTGAA-3′	5′-CGGACAATGTAGTCGAAGGAA-3′

COX, cyclooxygenase; cPGES and mPGES, cytosolic and microsonal prostaglandin E synthase.

and animals were refed. Fasted animals exhibited an increased consumption of food during the first few hours after the refeeding compared with animals fed ad libidum. Food intake was determined as described above.

RNA Extraction and RT-PCR

Total RNA was extracted from the frozen hypothalamus and DVC using a commercial reagent (TRIzol, Invitrogen) according to the manufacturer's instructions. The concentration and purity of the RNA were determined by the measurement of the optical density at 260 and 280 nm. Aliquots of 1 μg total RNA were used to generate the cDNA templates for PCR amplification. RT was performed using murine Moloney leukemia virus reverse transcriptase (200 units, Invitrogen) for 50 min at 37°C in the presence of random hexamer primers (Promega). The reaction was ended by heating the samples for 15 min at 70°C. The equivalent of 50 ng initial RNA was subjected to PCR amplification using *Taq* polymerase (1 units, Sigma; see Table 1 for oligonocleotides sequences and hybridizing temperatures). β-Actin was used as an internal standard. Thirty, thirty-two, and thirty-four cycles were performed for cytosolic PGES (cPGES) (rat, mouse), mPGES-1 (rat, mouse), and mPGES-2 (rat, mouse); 32, 34, and 36 cycles were performed for COX-1 (rat, mouse) and COX-2 (rat, mouse); and 26, 28, and 30 cycles were performed for β-actin. The amplicons were resolved by agarose gel (1%), and the fluorescence of resulting bands was quantified using a Bio-Rad Gel Doc 2000 apparatus. The log of the intensities of the bands was plotted as a function of the cycle number. Fluorescence values corresponding to a chosen cycle in the linear part of the curve were taken to express the results as follows: $[(F_X/F_{\beta\text{-actin}})$ treated/$(F_X/F_{\beta\text{-actin}})$ control], where F_X is the fluorescence of the amplicon of interest (X) and $F_{\beta\text{-actin}}$ is the fluorescence of the β-actin amplicon from the same sample.

Statistical Analysis

Comparisons between two groups were performed with Student's unpaired *t*-test. P values of <0.05 were considered significant. For each experiment, the test group comprised at least six animals. Mean $\pm$ SE values were derived from at least three independent experiments.

RESULTS

Effect of an Anorexic Dose of IL-1β on PGE₂-Synthesizing Enzyme Transcript Expression

To determine the consequences of IL-1β-induced inflammation on mPGES-1 expression in the hypothalamus and DVC, rats received an anorexic dose of IL-1 (16 μg/kg) by intraperitoneal injections at the beginning of their active period. Their food consumption and weight were measured at 3, 6, and 12 h after the injection. In agreement with previous studies, treatment with IL-1β induced a significant reduction in food intake associated with weight loss (Fig. 1). At each time of measurement, one group of animals was killed to determine the expression of the enzymes involved in PGE₂ synthesis (i.e., COX-1, COX-2, cPGES, mPGES-1, and mPGES-2) in the hypothalamus and DVC. At 3 and 6 h postinjection, no significant differences were found in the levels of COX-1, cPGES, and mPGES-2 transcripts between IL-1β- and saline-treated animals whatever the tissue considered (Figs. 2, *A* and *D–F*, and 3, *A* and *D–F*). Twelve hours after the injection, only a slight but significant decrease in COX-1 transcripts in the DVC was observed. In contrast, COX-2 and mPGES-1 mRNA were strongly upregulated 3 h after the injection in both the hypo-
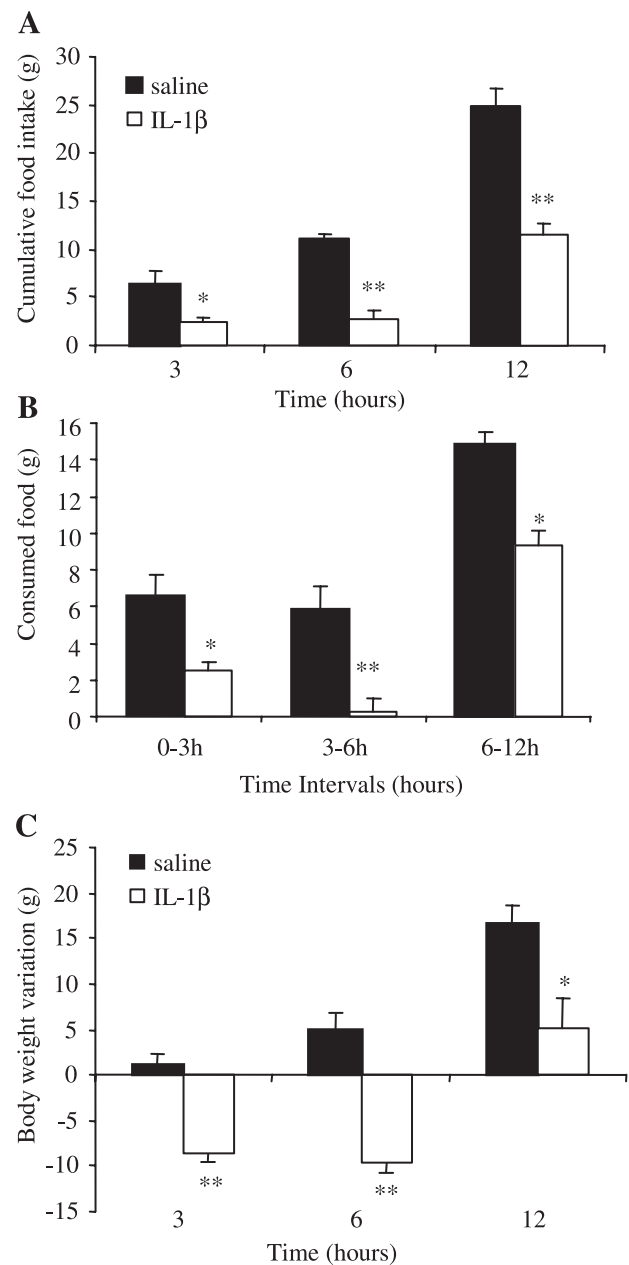

Fig. 1. Effects of an intraperitoneal injection of IL-1β on food intake and body weight. *A*: cumulative food intake measured 3, 6, and 12 h after either an intraperitoneal injection of saline or IL-1β (16 μg/kg) in adult rats. *B*: food consumed during the defined intervals of time, i.e., 0–3, 3–6, and 6–12 h, by rats that received either a saline or IL-1β (16 μg/kg) injection. *C*: body weight variation measured 3, 6, and 12 h after an intraperitoneal injection of saline or IL-1β (16 μg/kg) in adult rats. *P < 0.05 and **P < 0.01, significantly different from saline-treated rats.

thalamus (Fig. 2, *B, C,* and *F*) and DVC (Fig. 3, *B, C,* and *F*). Six hours after the injection, mPGES-1 gene expression remained higher in IL-1β-treated animals irrespective of the tissue analyzed. COX-2 transcript expression returned to the basal level as soon as 6 h in both structures analyzed (Figs. 2*B* and 3*B*). After 12 h, mPGES-1 transcripts remained significantly higher only in the DVC of IL-1β-treated animals (Fig. 3, *C* and *F*); their levels returned to the basal level in the hypothalamus (Fig. 2, *C* and *F*).
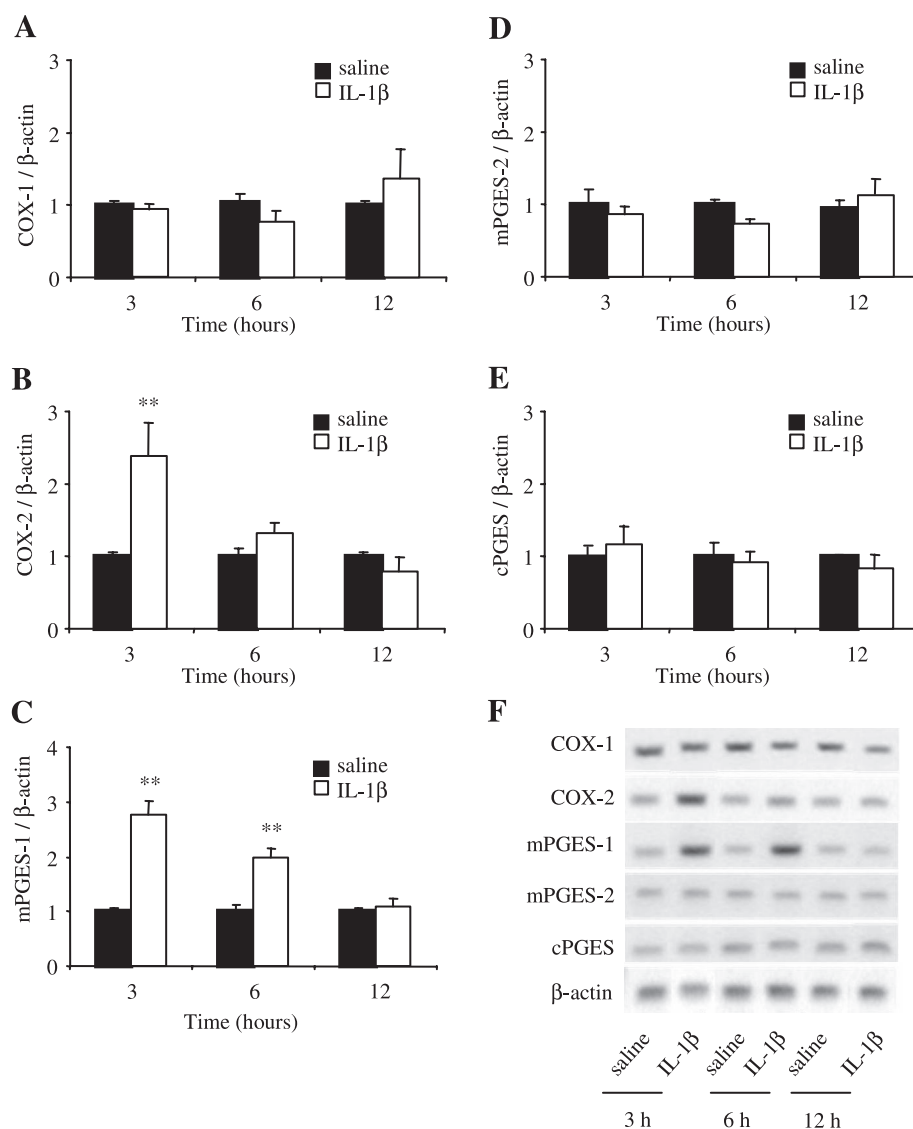
Fig. 2. Effects of an intraperitoneal injection of IL-1β on PGE$_2$-synthesizing enzyme mRNA expression in the hypothalamus. *A–E*: quantification of cyclooxygenase (COX)-1 (*A*), COX-2 (*B*), microsomal prostaglandin E synthase (mPGES)-1 (*C*), mPGES-2 (*D*), and cytosolic prostaglandin E synthase (cPGES; *E*) transcript expression in the hypothalamus 3, 6, and 12 h after the injection of saline- or IL-1β-containing solution. *F*: representative RT-PCR samples of PGE$_2$-synthesizing enzymes from the hypothalamus. *$P < 0.05$ and **$P < 0.01$, significantly different from saline-treated rats.

mPGES-1 Transcripts in the DVC and Hypothalamus After an Injection of IL-1β in the Fourth Ventricle

To determine whether centrally induced anorexia is also associated with an upregulation of mPGES-1 in the DVC and hypothalamus, we performed intracerebroventricular injections of IL-1β (10 ng/rat). The fourth ventricle was chosen as the site of injection. Severals reasons support this choice. The DVC is a caudal brain stem lining the fourth ventricle involved in the initiation of the meal reflex as well as in the satiety reflex. Several lines of evidences support the DVC as a gateway for immune messages from the periphery to higher brain regions (see the Introduction). Peripheral inflammation induces a robust c-Fos immunoreactivity within the DVC and the local production of cytokines (12). The pharmacological inhibition of the DVC prevents the activation of other higher brain nuclei, such as the hypothalamus, by intraperitoneal injections of proinflammatory molecules (14).

Intracerebroventricular injections of IL-1β (10 ng/rat) in the fourth ventricle induced a significant reduction in food intake in treated rats compared with control animals. The observed

hypophagia became significant 2 h after the injection (Fig. 4*B*) and lasted up to 6 h postinjection (data not shown). Furthermore, 1 h after the IL-1β injection, an increase in mPGES-1 transcripts was already observed in the DVC but not in the hypothalamus (Fig. 4, *C* and *D*). Three hours after the injection, during the ongoing anorexia, mPGES-1 transcripts levels were increased in both structures (Fig. 4, *C* and *D*).

Treatment of mPGES-1$^{-/-}$ Mice by an Anorexic Dose of IL-1β

To determine whether upregulation of the mPGES-1 gene may account for the hypophagia and weight loss observed after IL-1β-induced inflammation, we evaluated the impact of such a treatment on mPGES-1-deficient (mPGES-1$^{-/-}$) mice. After a period of fasting, food intake was quantified for mPGES-1$^{-/-}$, heterozygous, and wild-type mice treated intraperitoneally with either 1 μg IL-1β or physiological saline. The quantification of food intake over a period of 5 h after the saline injection did not reveal any difference in the feeding behavior of mPGES-1$^{-/-}$, heterozygous, and wild-type mice
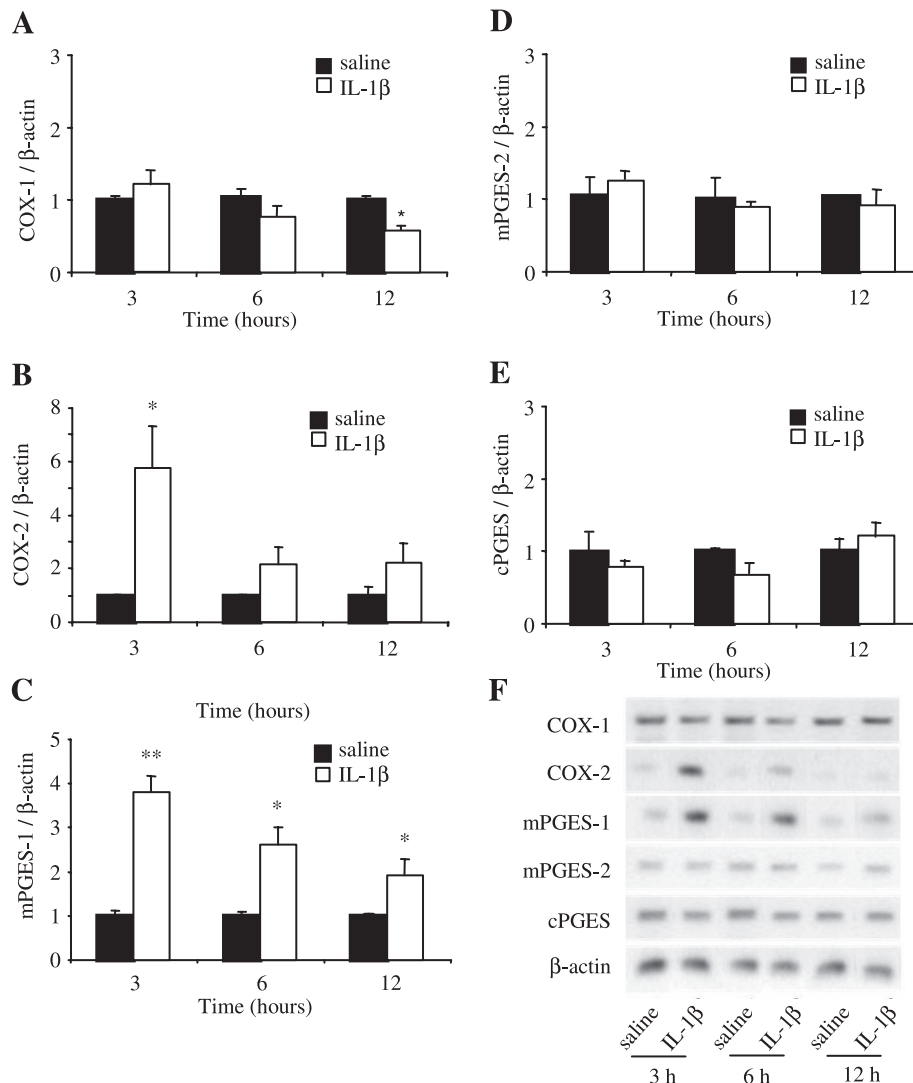
Fig. 3. Effects of an intraperitoneal injection of IL-1β on PGE$_2$-synthesizing enzyme mRNA expression in the dorsal vagal complex (DVC). A–E: quantification of COX-1 (A), COX-2 (B), mPGES-1 (C), mPGES-2 (D), and cPGES (E) transcript expression in the DVC 3, 6, and 12 h after the injection of saline- or IL-1β-containing solution. F: representative RT-PCR samples of PGE$_2$-synthesizing enzymes from the DVC. *$P < 0.05$ and **$P < 0.01$, significantly different from saline-treated rats.

(Fig. 5, A–C). In response to an intraperitoneal IL-1β injection, heterozygous and wild-type mice exhibited a significant inhibition of food consumption (~30%; Fig. 5, A and B). In contrast, IL-1β-treated mPGES-1$^{-/-}$ mice displayed no significant differences in the quantity of food consumed compared with saline-injected mice (Fig. 5C). By RT-PCR, we confirmed the induction of mPGES-1 in the hypothalamus and DVC of IL-1β-treated mPGES-1 wild-type and heterozygous mice as well as the absence of mPGES-1 in mPGES-1$^{-/-}$ mice (Fig. 5E). Interestingly, we also showed that COX-2 was strongly upregulated by IL-1β treatment in both the DVC and hypothalamus of mPGES-1$^{-/-}$ mice, as observed in wild-type mice (Fig. 5E).

Treatment of mPGES-1$^{-/-}$ Mice by an Anorexic Dose of PGE$_2$

To rule out the possibility that PGE$_2$ signaling pathway defects could explain the lack of anorexia observed in mPGES-1$^{-/-}$ mice, we studied the feeding behavior of mPGES-1$^{-/-}$ mice after an intraperitoneal PGE$_2$ injection. The reduction in food intake induced by injections of anorexic doses of PGE$_2$ was similar in mPGES-1$^{-/-}$ and wild-type mice (Fig. 6). As

soon as 30 min, PGE$_2$ suppressed the absorption of food of mPGES-1$^{-/-}$ and wild-type mice fasted during 16 h. One hour after injections, the food intake remained strongly reduced in mice injected with PGE$_2$ compared with saline-treated mice. Two and three hours after injections, the cumulative food intake of PGE$_2$-treated mice remained significantly lower than that observed in controls, albeit food consumed during the last 2 h was quite higher in both PGE$_2$-treated groups than in controls (Fig. 6).

DISCUSSION

In the present study, we showed that intraperitoneal injections of IL-1β able to decrease significantly food intake induce an upregulation of COX-2 and mPGES-1 in the DVC and hypothalamus, two central structures strongly involved in the regulation of food intake. The time course of mPGES-1 regulation in these structures suggests that this enzyme could be instrumental in hypophagic behavior. Indeed, the results reveal that 3 h after a peripheral IL-1β injection, COX-2 and mPGES-1 transcripts were strongly upregulated in both central structures while the hypophagic behavior was ongoing. Six hours after the IL-1β injection, mPGES-1 upregulation per-
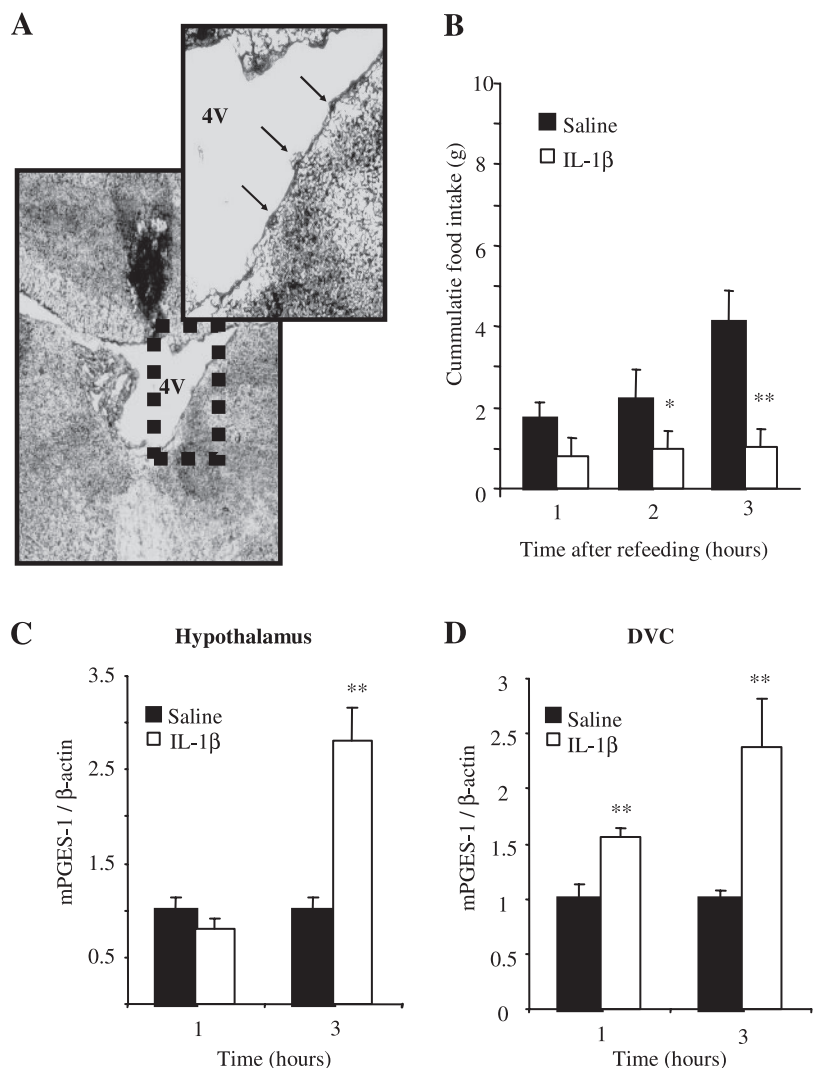
Fig. 4. Effects of an intracerebroventricular injection of IL-1β on food intake and mPGES-1 mRNA expression. *A*: section of the rat brain showing the sites of the injections at the fourth ventricle (4V). *Inset*, higher magnification view with the arrows showing the injection sites. *B*: food intake was measured over 1–3 h after the injection of IL-1β (10 ng/kg) or saline into the 4V. *C* and *D*: mPGES-1 mRNA in the DVC (*C*) and hypothalamus (*D*) was quantified 1 and 3 h after the injection of IL-1β. *$P < 0.05$ and **$P < 0.01$, significantly different from saline-treated rats.

sisted in the DVC and hypothalamus, whereas the expression of COX-2 transcripts returned to the basal level. Finally, 12 h after the IL-1β injection, animals displayed lower but still significant hypophagic behavior and mPGES-1 upregulation was only observed in the DVC. This sustained mPGES-1 upregulation in brain stem may participate in the reduction in food intake observed at the longer times after the IL-1β injection. Such a mechanism has been previously suggested by a time course study of c-Fos mRNA and protein expression in the hypothalamus and brain stem after an intraperitonal injection of IL-1β. In this report (18), the persistent increase in c-Fos expression in brain stem/gut recipient sites, as the nucleus of the solitary tract, was proposed to participate in the belated hypophagia. However, the mPGES-1 mRNA analysis was obtained from the whole hypothalamus, and, in the future, it will be of interest to determine whether peripheral IL-1β injection induces differential expression of mPGES-1 in different hypothalamic nuclei.

Intraperitoneal injections of IL-1β may result in the induction of PG-synthesizing enzymes throughout the brain; we performed intracerebroventricular injections of the cytokines to confine stimulation to the structures lining the ventricles. We showed that IL-1β injected in the fourth ventricle resulted in a reduction of food intake that became significant 2 h after the injection. Moreover, this hypophagic behavior was long lasting and still present 6 h after the injection. This hypophagic dose of centrally injected IL-1β induced a strong rise in mPGES-1 transcripts in both the DVC and hypothalamus. Interestingly, mPGES-1 transcripts increased after 1 h in the DVC but not in the hypothalamus, where an upregulation of mPGES-1 was observed 3 h after the central injection. This rapid induction of mPGES-1 in the DVC should be compared with the onset of anorexia, which took place 2 h after the injection. These results strengthen the hypothesis of a role of central induction of mPGES-1 in initiating anorexia. Finally, to clearly demonstrate the link between anorexia and mPGES-1 expression, we studied the IL-1β-induced hypohagia in mPGES-1$^{-/-}$ mice. Interestingly, these animals did not exhibit hypophagic behavior in response to IL-1β, contrary to the heterozygous and wild-type mice. In addition, an intraperitoneal injection of PGE₂ induced rapid anorexia in both mPGES-1$^{-/-}$ and wild-type mice, strongly suggesting that the absence of anorexia after IL-1β injections is not due to a failure in PGE₂ signaling.

The present report extends previous studies (7, 13, 23, 24) in which the involvement of the PG pathway in anorexia observed after inflammatory challenge was proposed. Indeed, COX-2
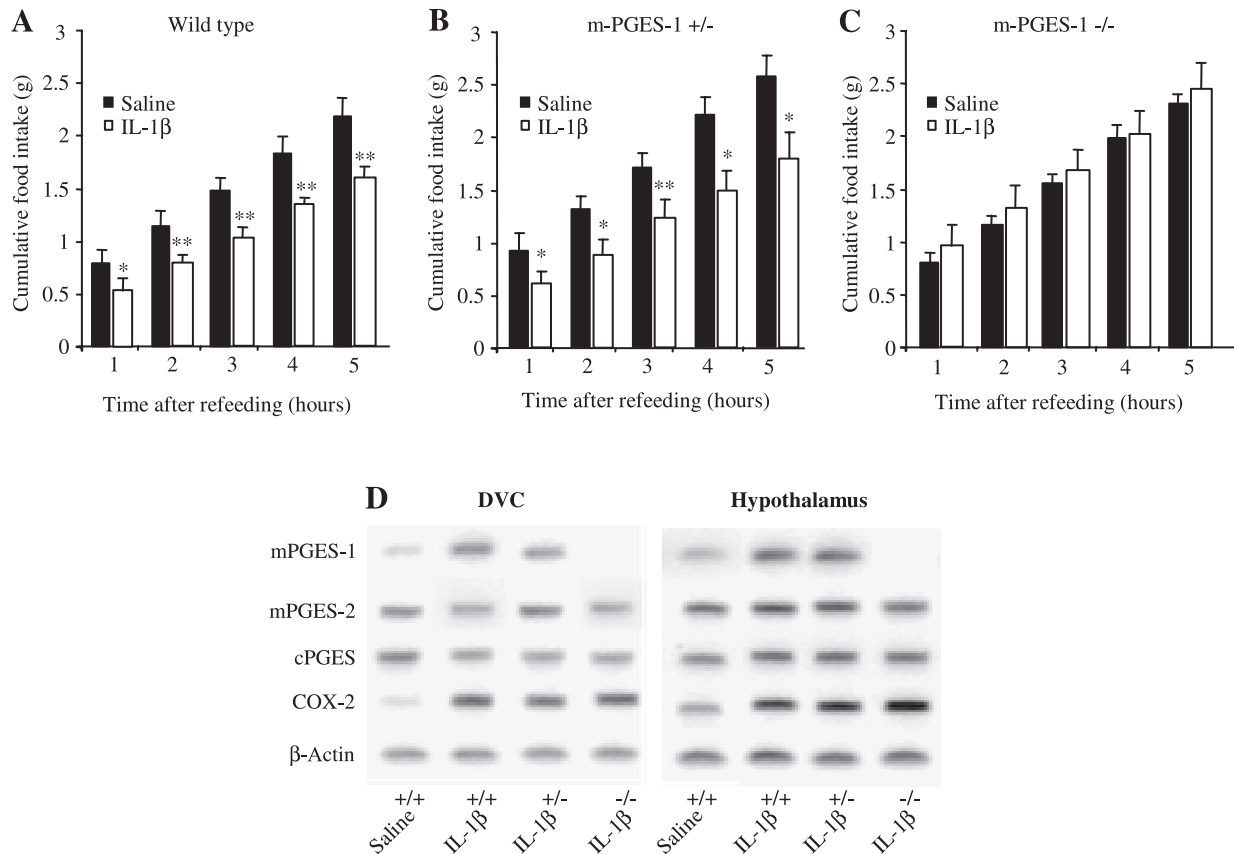
Fig. 5. Effects of an intraperitoneal injection of IL-1β on mPGES$^{-/-}$, mPGES$^{+/-}$, and wild-type (mPGES-1$^{+/+}$) mouse food intake, weight, and PGE$_2$-synthesizing enzyme mRNA expression. Food intake of wild-type (A), mPGES-1$^{+/-}$ (B), and mPGES-1$^{-/-}$ (C) mice was measured over a period of 5 h after an intraperitoneal injection of saline or IL-1β (1 μg/mouse). D: representative RT-PCR samples of PGE$_2$-synthesizing enzyme mRNA expression in the DVC and hypothalamus after either a saline or IL-1β injection in wild-type, mPGES-1$^{+/-}$, and mPGES-1$^{-/-}$ mice. *$P < 0.05$ and **$P < 0.01$, significantly different from saline-treated mice.

knockout mice exhibited less weight loss than wild-type mice measured 1 and 2 days after LPS challenge (7), suggesting that COX-2 partakes in the hypophagic behavior. However, in the absence of food intake measurements in this study, we cannot exclude the fact that modifications in energy expenditure contributed to the attenuation of weight loss associated with COX-2 deficiency, because COX-2 was demonstrated to par-
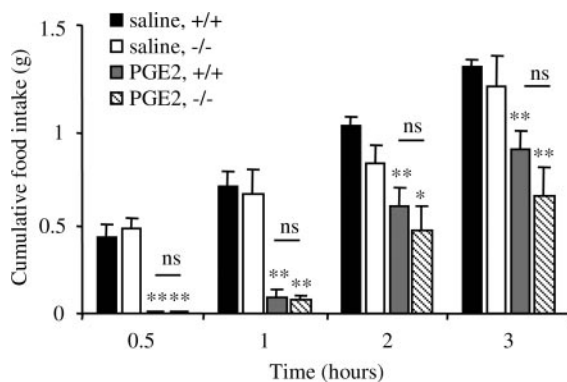

Fig. 6. Effects of an intraperitoneal injection of PGE$_2$ (20 μg/mouse) on the feeding behavior of mPGES-1$^{-/-}$ and mPGES-1$^{+/+}$ mice. Cumulative food intake was measured 0.5, 1, 2, and 3 h after either an intraperitoneal injection of saline or PGE$_2$ (20 μg/animal) in adult rats. *$P < 0.05$ and **$P < 0.01$, significantly different from saline-treated mice. ns, Not significant.

ticipate in the febrile response (29). Nevertheless, the anorexic action of LPS and IL-1β has been shown to be attenuated by indomethacin, a nonspecific inhibitor of PG synthesis, and by more specific COX-2 inhibitors (7, 13). Moreover, Lugarini and colleagues (13) reported that doses of COX-2 inhibitors that reduce anorexic effect of LPS abolished the cytokine-induced PGE$_2$ increase observed in CSF. The authors also observed that low doses of COX-2 inhibitors failed to attenuate anorexia while still being able to suppress the LPS-induced PGE2 increase in CSF (13). The involvement of different arachidonic acid metabolites was thus proposed by the authors to contribute to the feeding reduction observed during IL-1β or LPS treatment, because other PGs (i.e., PGF$_2$) were shown to display anorexic activity when administered centrally (11). However, mPGES-1-null mice did not exhibit anorexia in response to IL-1β, whereas, in these animals, the reduction of LPS-induced PGE$_2$ formation is balanced by an increased production of thromboxane B$_2$, PGF$_1$, and PGF$_2$ (26). The apparent discrepancy could reflect a possible difference between the PGE$_2$ concentration in CSF and the local level of this eicosanoid in specific brain structures such as the hypothalamus and caudal brain stem.

In the present study, we observed that despite the strong upregulation of the COX-2 gene in the hypothalamus and DVC of mPGES-1$^{-/-}$ mice, these animals did not develop anorexia

in response to IL-1β. The results strongly suggest that the induction of COX-2 is not sufficient to increase PGE$_2$ at a level able to produce anorexia despite the presence of the two noninducible PGES, i.e., cPGES and mPGES-2. Hence, mPGES-1-catalyzed PGE$_2$ production appears necessary to induce anorexic behavior. Our results demonstrate that mPGES-1 is involved in inflammatory-induced anorexia as previously demonstrated for fever by Emgblom and colleagues (3). However, it should be noted that, although fever and anorexia are both induced during infection and inflammation and related to mPGES-1 expression, they are at least partially dissociable events. Indeed, numerous studies (10, 17, 27) have clearly demonstrated the lack of correlation between the direction and timing of temperature changes and the reduction of feeding behavior in different models of peripheral inflammation.

In conclusion, our results point to mPGES-1 and PGE$_2$ as crucial mediators in anorexia in response to peripheral infection and neuroinflammation and suggest that specific inhibition of mPGES-1 may constitute a potential therapeutic strategy for the treatment of this sickness behavior, particularly because a recent study (8) has postulated that selective inhibition of COX-2 may be also detrimental. Numerous studies from different groups have suggested that PGE$_2$ is also involved in cancer-related cachexia development (28). Given our results, it appears critical to determine whether mPGES-1 expression is modulated in central structures of tumor-bearing animals and whether genetic ablation of mPGES-1 could prevent or diminish the reduction of appetite observed in these pathological conditions.

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