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Systemic lipopolysaccharide influences rectal sensitivity in rats: role of mast cells, cytokines, and vagus nerve

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Coelho, Anne-Marie, Jean Fioramonti, and Lionel Buéno. Systemic lipopolysaccharide influences rectal sensitivity in rats: role of mast cells, cytokines, and vagus nerve. *Am J Physiol Gastrointest Liver Physiol* 279: G781–G790, 2000.—Intraperitoneal lipopolysaccharide (LPS) produces somatic hyperalgesia, releases interleukin (IL)-1 β and tumor necrosis factor- α (TNF- α), and activates vagal afferents. The aim of this study was to evaluate the effect of peripheral LPS on rectal sensitivity and to specify the mechanisms involved. Abdominal muscle contractions were recorded in conscious rats equipped with intramuscular electrodes. Rectal distension (RD) was performed at various times after LPS or experimental treatments. In controls, RD significantly increased the number of abdominal contractions from a threshold volume of distension of 0.8 ml. At the lowest volume (0.4 ml), this number was increased after administration of LPS (3, 9, and 12 h later), recombinant human IL-1 β (from 3 to 9 h), recombinant bovine TNF- α (from 6 to 9 h), and BrX-537A (from 6 to 12 h), a mast cell degranulator. The effect of LPS was reduced by doxantrazole, Lys-D-Pro-Thr, and soluble recombinant TNF receptor. Vagotomy selectively amplified the response to LPS. We conclude that, in vivo, intraperitoneal LPS lowers visceral pain threshold (allodynia) through a mechanism involving mast cell degranulation and IL-1 β and TNF- α release and that the vagus nerve may exert a tonic protective role against LPS-induced rectal allodynia.

endotoxins; rectal allodynia; mast cells; interleukin-1 β ; tumor necrosis factor- α ; subdiaphragmatic vagotomy

THE GASTROINTESTINAL TRACT represents one of the body's largest interfaces with the outside environment. It possesses a complex immune system providing its defense against environmental threats including infection by viruses, bacteria, and parasites. Approximately one-third of patients with bacterial gastroenteritis develop chronic abdominal symptoms and signs of sensory changes in the gut (1). This entity, called "postinfectious irritable bowel syndrome" (PI-IBS), accounts for ~30% of all irritable bowel syndrome (IBS) patients. The major symptoms observed in patients with IBS include disordered colonic motility and acute or chronic abdominal pain. These patients exhibit an exaggerated sigmoid motor response to a variety of stimuli (41) and also have a lowered visceral sensory threshold to pain caused by balloon distension (35).

Unfortunately, the pathophysiology of visceral hypersensitivity in patients with both IBS and bacterial infection is not precisely known.

Lipopolysaccharide (LPS), also known as endotoxin, is a cell wall Gram-negative bacteria component. It induces a wide array of effects after bacterial infection, including fever (18), sickness behavior (17), and hyperalgesia. Indeed, several studies have shown that an intraperitoneal injection of LPS enhances pain responsiveness to various somatic stimuli (see Refs. 43 and 46). Moreover, LPS-induced alterations in nociception depend on proinflammatory cytokines released from monocytes and macrophages under LPS stimulation such as interleukin (IL)-1, IL-6, and tumor necrosis factor- α (TNF- α). Indeed, an important role of inflammatory cytokines at the peripheral level has been recently recognized in sensory hypersensitivity (44). For example, cutaneous hyperalgesia can be produced by intraperitoneal injection of IL-1 (22) and TNF- α (42). Moreover, after peripheral administration, LPS-, IL-1 β -, and TNF- α -induced hyperalgesia requires vagal integrity because it is blocked by subdiaphragmatic vagotomy (43, 44). This result agrees with the observation that brain release of proinflammatory mediators, including cytokines, is mediated in part by vagal afferents (19), even though it does not appear to be the only route for LPS/cytokine-to-brain communication (10).

Kanaan et al. (15) showed that intraplantar injection of endotoxin produces local inflammation and delayed somatic hyperalgesia, mediated locally by IL-1 β , nerve growth factor (NGF), and PGE₂ (39). This hyperalgesic effect starts 1–2 h after intraplantar endotoxin injection and peaks at 9 h in rats and 24 h in mice (16). Similarly, we recently reported (3) that experimental mast cell degranulation in vivo induces a delayed (6–12 h) increase in sensitivity (allodynia) to rectal distension in awake rats. Indeed, mast cells are involved in postinfectious (24) and stress-induced (13) hyperalgesia, and their density is altered in functional bowel disorders where, for example, an accumulation of mast cells in the ileum has been demonstrated (45). The anatomic arrangement of mast cells places them in the

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first line of defense against injury or infection, particularly for skin, airways, and gastrointestinal tract, sites that interface directly with the external environment. These cells are well suited to initiate an acute inflammatory process and, through interaction with other tissue cells, to continue to maintain or modulate later response.

No studies have investigated the influence of systemic LPS on visceral sensitivity, as was previously established for somatic sensitivity. Consequently, the present study was designed to evaluate whether intraperitoneal administration of endotoxin can initiate visceral allodynia to rectal distension in rats and to determine the role of peripheral IL-1 β and TNF- α , the involvement of mast cells, and the neuronal (vagus and/or other) pathway in LPS-related allodynia.

MATERIALS AND METHODS

General Surgical Procedure

Animal preparation. Male Wistar rats (Harlan, Gannat, France), initially weighing between 200 and 250 g, were surgically prepared for electromyography according to a previously described technique (38). Rats were anesthetized by intraperitoneal injection of acepromazine (Calmivet, Vetoquinol, Lure, France) and ketamine (Imalgene 1000, Rhône-Mérieux, Lyon, France) at doses of 0.6 and 120 mg/kg, respectively. Three groups of three electrodes of NiCr wire (60-cm length and 80-mm diameter) were implanted bilaterally in the abdominal external oblique musculature just superior to the inguinal ligament. Electrodes were exteriorized on the back of the neck and protected by a glass tube attached to the skin. Animals were individually housed in polypropylene cages and kept in a temperature-controlled room (21°C). They were allowed free access to water and food (UAR pellets, Epinay, France). All protocols were approved by the Local Animal Care and Use Committee of Institut National de la Recherche Agronomique.

Electromyographic recording. Electromyographic recordings began five days after surgery. The electrical activity of abdominal striated muscles was recorded with an electroencephalograph machine (Mini VIII, Alvar, Paris, France) using a short time constant (0.03 s) to remove low-frequency signals (<3 Hz) and a paper speed of 3.6 cm/min.

Distension procedure. Rats were placed in plastic tunnels (6-cm diameter and 25-cm length) in which they could not move, escape, or turn around, to prevent damage to the balloon. Rats were exposed to this procedure over 3 days before rectal distension (RD) to minimize stress reactions during experiments. The balloon used for distension was an arterial embolectomy catheter (Fogarty, Edwards Laboratories, Santa Ana, CA). RD was performed by insertion of the balloon (2-mm diameter and 2-cm length) in the rectum, at 1 cm of the anus, the catheter being fixed at the tail with adhesive tape. The balloon was inflated progressively, in 0.4-ml steps, from 0 to 1.6 ml, each step of inflation lasting 5 min. To detect possible leakage, the volume of water introduced into the balloon was checked by complete removal with a syringe at the end of the distension period.

Temperature Recording

To measure body temperature (T_b), a thermistor probe (NTC type, code 10K3A1, Farnell, Villefranche sur Saône, France) was placed in the peritoneal cavity, using a previ-

ously described technique (26). T_b was recorded 5 days after surgery by connecting the thermistor probe to an electronic thermometer developed in our laboratory. It was calibrated to give an initial output of 0 V at 35°C with a sensitivity of 200 mV/°C. The temperature was monitored on a potentiometric recorder (L6514, Linseis, Selb, Germany) with a paper speed of 2 cm/h.

Subdiaphragmatic Vagotomy

Surgery. Seven days before implantation of abdominal electrodes, abdominal vagotomies were performed as follows. Rats were anesthetized with ketamine and acepromazine (120 and 0.6 mg/kg ip, respectively). After midline laparotomy, the stomach and lower esophagus were visualized. The stomach was gently retracted beneath the diaphragm to clearly expose the ventral and dorsal trunks of the vagus nerve and covered with saline-moistened sterile gauze. Each vagal trunk was dissected from the esophagus and sectioned. The stomach was then returned to its normal position, and the incision was closed. Sham vagotomies consisted of the same operative procedure except that the vagal trunks were neither tied nor sectioned. Animals were returned to their home cages after the operation and were provided with food and water ad libitum. Normal food intake resumed within 3–5 days after vagotomy.

Verification procedure. The effectiveness of vagus nerve section was assessed 14 days after vagotomy by the sulfated cholecystokinin (CCK-8S) satiety test. Subdiaphragmatic vagotomy suppresses the blockade of food consumption induced by CCK-8S. Consequently, CCK-8S or saline was injected at the dose of 4 μ g/kg ip after 20 h of food deprivation, and food intake was measured 1 h after injection.

Histological Mast Cell Counting Method

Intestinal tissue samples were put in Carnoy's solution immediately after the animals were killed, fixed for 24 h at room temperature, and then embedded in paraffin blocks using routine techniques. Sections were cut at a thickness of 5 mm and stained with hemalun-eosin for routine histological analysis or with Alcian blue-Safranin O for identification of intestinal mast cells. Three sections for each sample and each animal were analyzed by light microscopy, and the number of intact mast cells was counted for each section. For each animal, the number of intact mast cells per square millimeter of intestinal tissue was the mean of the values obtained for the three sections.

Chemicals

LPS (from *Escherichia coli*, serotype 0111:B4; L3024, lot no. 38H4065) was purchased from Sigma-Aldrich (St. Quentin Fallavier, France) and was dissolved in saline (NaCl 0.9%) at a concentration of 1 mg/ml. BrX-537A (bromolasalocid ethanolate) was kindly supplied by Roche Laboratories (London, UK) and was dissolved in DMSO at a concentration of 2 mg/ml. Doxantrazole was obtained from Wellcome Research Laboratories (lot no. 59C72, Beckenham, UK) and was dissolved in DMSO (5 mg/ml). Recombinant human IL-1 β (rhIL-1 β) and recombinant human dimeric soluble TNF receptor (rhuTNF-Fc molecule p75, linked to the Fc portion of the human IgG1; sTNFR) were kindly provided by Dr. Michael B. Widmer (Immunex, Seattle, WA). They were dissolved in saline and Tris-NaCl, pH 7.4, respectively. The construction and production of sTNFR has been previously described (27). Recombinant bovine TNF- α (rboTNF- α) was kindly provided by Sandoz (Basel, Switzerland) and dis-

solved in saline at a final concentration of 150 $\mu\text{g/ml}$. The tripeptide H-Lys-D-Pro-Thr-OH was purchased from Bachem AG (H-7230, lot no. 122401, Budendorf, Switzerland) and dissolved in saline at a concentration of 10 mg/ml . In all experiments, intraperitoneal injections of drug or vehicle were given in a volume of 1 ml/kg .

Experimental Protocol

Effect of LPS and role of mast cells. These studies determined the effects of intraperitoneal injection of LPS on rectal sensitivity and the involvement of mast cells using both pharmacological and histological methods. In a first series of experiments, two groups of rats were used. In the first group ($n = 6-8$), rats were injected intraperitoneally with BrX-537A (mast cell degranulator; 2 mg/kg ip). RDs were performed before (-1 h, control) and 3, 6, 9, 12, and 24 h after BrX-537A or vehicle administration. The dose of BrX-537A (2 mg/kg) has been found active in a model of rectal sensitivity (3). Eight days later, the same animals were injected intraperitoneally with LPS vehicle (saline) and, 48 h later, received LPS (1 mg/kg ip). RDs were performed before (-1 h, control) and 3, 6, 9, 12, and 24 h after LPS or vehicle administration. The dose of LPS was chosen according to preliminary experiments showing less reproducible and significant data at lower doses (i.e., 0.1 and 0.5 mg/kg ip; Coelho et al., unpublished observations). In the second group ($n = 8$), rats were injected, in a randomized order, with doxantrazole (5 mg/kg ip), a mast cell stabilizing agent, or its vehicle 20 min before LPS or its vehicle. RDs were performed before (-1 h, control) and 3, 6, 9, and 12 h after LPS administration. An 8-day interval was observed between two single LPS administrations. The dose of doxantrazole (5 mg/kg ip) was chosen according to its efficacy in preventing mast cell degranulation-induced rectal allodynia (3). The time chosen between two single administrations of LPS (8 days) was judged to be the minimum necessary time for a complete recovery from each LPS treatment to eliminate a tolerance parameter of our LPS treatments.

In a second series of experiments, five groups of eight male Wistar rats weighing 250–300 g were used for histological studies. *Group 1* was used as control. *Groups 2* and *3* received BrX-537A (2 mg/kg ip). *Groups 4* and *5* received LPS (1 mg/kg ip). Rats in *groups 2* and *4* were killed 1 h after administration; those in *groups 3* and *5* were killed 5 h later. Tissue samples of ileum and proximal colon (2–3 cm from the cecocolonic junction) were collected and prepared for mast cell counting.

Effect of LPS on T_b and behavior. T_b was recorded in a group of five rats. On *day 1*, animals were injected intraperitoneally with vehicle (saline, 1 ml/kg) after 1 h of temperature control recording. Temperature was monitored for a period of 24 h to establish a baseline. On *day 2*, the same animals were injected intraperitoneally with LPS (1 mg/kg) and T_b was recorded for 24 h. All measurements were performed at a subthermoneutral ambient temperature of $21.0 \pm 1.0^\circ\text{C}$. All animals received saline or LPS between 8:30 and 9:00 AM.

Role of IL-1 β . Two groups of rats were used to evaluate the role of IL-1 β . *Group 1* ($n = 9$) was given rhIL-1 β at a dose (10 $\mu\text{g/kg}$ ip) known to induce sickness behavior (44). Vehicle was injected intraperitoneally for control purposes. RDs were performed 3, 6, 9, and 12 h after rhIL-1 β or vehicle administration. *Group 2* ($n = 5$) received, in a randomized order, intraperitoneal injection of tripeptide Lys-D-Pro-Thr (or vehicle) 30 min before LPS injection, at the dose (10 mg/kg)

known to antagonize IL-1 β -induced hyperalgesia (7) and to significantly reduce the hyperalgesic effect of intraplantar LPS (39). The same group received an injection of tripeptide alone. RDs were performed before (-1 h, control) and 12 h after LPS (or vehicle) injection. Eight days separated LPS/vehicle and LPS/tripeptide randomized treatments.

Role of TNF- α . Two groups of rats were used. *Group 1* ($n = 6$) was injected intraperitoneally with rboTNF- α , or its vehicle, at a dose (150 $\mu\text{g/kg}$) found to be active in a model of somatic hyperalgesia (42). RDs were performed 3, 6, 9, and 12 h later. *Group 2* ($n = 6$) was injected twice intraperitoneally with sTNFR (total dose 2 mg/kg) or vehicle; the first injection (1 mg/kg) was performed immediately before LPS (1 mg/kg ip) or saline, and the second injection (1 mg/kg) was performed 90 min later. RDs were performed before (-1 h, control) and 12 h after LPS or saline injection. The delay of 90 min for the second injection was chosen according to the efficacy of sTNFR, a procedure previously validated in mice (27). Eight days separated LPS/vehicle and LPS/sTNFR randomized treatments.

Effect of subdiaphragmatic vagotomy on LPS effect. Four groups of rats were used to determine the role of vagal nerves: sham vagotomy + vehicle ($n = 5$), sham vagotomy + LPS ($n = 8$), vagotomy + vehicle ($n = 7$), and vagotomy + LPS ($n = 7$). Rats were injected intraperitoneally with LPS (1 mg/kg) or its vehicle. RD was performed 12 h after vehicle or LPS injection.

Statistical Analysis

Statistical analysis of the number of abdominal contractions for each 5-min period during RD was performed by one-way ANOVA followed by Student's unpaired or paired *t*-test where relevant. Values are expressed as means $\pm$ SE. T_b values are presented as means $\pm$ SE and were compared by one-way ANOVA followed by Student's paired *t*-test. Mast cell numbers per square millimeter were analyzed using the Mann-Whitney *U*-test for unpaired data, and values are expressed as means $\pm$ SE. All differences were considered significant at $P < 0.05$.

RESULTS

Effect of Intraperitoneal Injection of LPS on Rectal Sensitivity

Gradual RD increased the frequency of abdominal contractions in a distension volume-dependent manner. A volume of 0.8 ml was determined as the threshold at which RD induced a significant increase of the number of abdominal contractions compared with the predistension level (29). Saline-treated rats and untreated controls responded similarly to RD regardless of volume (0–1.6 ml) and time of distension (3, 6, 9, 12, and 24 h). LPS (1 mg/kg ip) increased the number of abdominal contractions for the 0.4-ml volume 3 (11.0 ± 1.8 contractions/5 min), 9 (10.5 ± 2.5 contractions/5 min), and 12 (16.1 ± 3.0 contractions/5 min) h after its administration compared with 3.4 ± 0.9 contractions/5 min for the control RD performed 1 h before LPS (Fig. 1). At other times (6 and 24 h) and other volumes (0.8–1.6 ml), abdominal responses were unaffected ($P > 0.05$) by LPS treatment (data not shown). On the basis of these data, we used the time of 12 h (maximal effect) to perform RDs in subsequent pharmacological

Fig. 1. Kinetics of lipopolysaccharide (LPS; A) and BrX-537A (B) effects on abdominal responses to rectal distension at the 0.4-ml volume in awake rats. Both LPS (1 mg/kg ip) and BrX-537A (2 mg/kg ip) increased the number of abdominal contractions elicited by the lowest volume of distension at 3, 9, and 12 h and 6, 9, and 12 h after injections, respectively. Values are means $\pm$ SE; $n = 6-8$ rats. Significant difference from control value measured 1 h before injections: * $P < 0.05$, ** $P < 0.01$.

investigations except for kinetic studies and determination of mast cell involvement.

Effect of Intraperitoneal Injection of LPS on T_b and Behavior

The basal core temperature of rats was $37.8 \pm 0.2^\circ\text{C}$. Intraperitoneal injection of vehicle (saline, 1 ml/kg) did not significantly ($P > 0.05$) modify the profile of T_b during the daytime period of recording. All rats displayed normal circadian changes in T_b , with lower daytime and higher nighttime T_b values (data not shown). LPS (1 mg/kg ip) significantly increased ($P <$

0.05) T_b between 1.5 and 9 h after injection. The T_b increase was characterized by the occurrence of two peak elevations, a first maximal T_b rise that peaked 2 h later (38.5 ± 0.2 vs. $37.5 \pm 0.1^\circ\text{C}$ of vehicle control) and a second maximal T_b rise that appeared 5.5 h later (38.4 ± 0.2 vs. $37.2 \pm 0.1^\circ\text{C}$) (Fig. 2). Between 10 and 24 h, there were no differences in T_b between vehicle and LPS treatments. Concerning behavioral effects, rats injected with 1 mg/kg LPS showed a few signs of illness such as piloerection and lack of activity, but these did not last longer than 24 h. At the high dose used, no deaths were noted.

Involvement of Mast Cells in LPS-Induced Rectal Hypersensitivity

As previously described (3), the number of abdominal contractions observed at the lowest volume of distension (0.4 ml) was significantly increased 6, 9, and 12 h after BrX-537A (2 mg/kg ip; 7.7 ± 2.0 , 8.2 ± 1.8 , and 13.1 ± 1.4 contractions/5 min, respectively, vs. 2.4 ± 0.5 for control RD) (Fig. 1). Doxantrazole (5 mg/kg ip) or its vehicle was given 20 min before LPS, and RD were performed 3, 6, 9, and 12 h after LPS. As in the previous series of experiments, animals receiving LPS after vehicle treatment showed an increase in the number of abdominal contractions only for the threshold volume of 0.4 ml at 3, 9, and 12 h after LPS (Fig. 3). Prior administration of doxantrazole (5 mg/kg ip) suppressed the abdominal response observed 3–12 h after LPS; compared with vehicle, doxantrazole significantly ($P < 0.05$) decreased the number of abdominal contractions at the 0.4-ml volume of distension, 3 (3.7 ± 1.7 vs. 11.0 ± 1.8 contractions/5 min for LPS + vehicle control) and 12 (5.7 ± 2.2 vs. 16.1 ± 3.0) h after LPS administration (Fig. 3).

Fig. 2. Effect of LPS on body temperature. LPS (1 mg/kg ip) induced a biphasic fever with 2 maximal peritoneal temperature rises, 2 and 5–6 h after injection, respectively. Values are means $\pm$ SE; $n = 5$ rats. *Significant difference ($P < 0.05$) from corresponding vehicle values.

Fig. 3. Effect of the mast cell stabilizer doxantrazole on LPS-induced increase of abdominal contractions in response to rectal distension (0.4 ml). Doxantrazole (5 mg/kg ip) reduced the increase in the number of abdominal contractions induced by LPS 3 and 12 h after injection. Values are means $\pm$ SE; $n = 8$ rats. Significant difference from the control value (-1 h): * $P < 0.05$, ** $P < 0.01$. †Significant difference ($P < 0.05$) from corresponding LPS + vehicle value.

Mast Cell Numbers After LPS Administration

To confirm the involvement of mast cells in LPS-induced visceral hypersensitivity and to compare the time course of mast cell degranulation with BrX-537A, histological examination of mucosal mast cells in the ileum and proximal colon was performed on rats treated with either BrX-537A or LPS. The number of intact mucosal mast cells in control animals was 215.1 ± 33.1 and 133.0 ± 17.9 cells/mm² in the ileum and proximal colon, respectively. When rats were injected with BrX-537A (2 mg/kg ip), this number was significantly reduced (107.2 ± 17.6 and 81.2 ± 38.9 cells/mm²; $P < 0.05$) 1 h after BrX-537A administration. In contrast, rats given LPS presented a lower number of intact mucosal mast cells only 5 h (131.0 ± 22.3 and 72.8 ± 23.7 cells/mm²) after LPS administration (Table 1).

Table 1. Number of intact mast cells identified in ileum and proximal colon 1 and 5 h after BrX-537A or LPS administration

	Control	BrX-537A (2 mg/kg ip)		LPS (1 mg/kg ip)	
		+1 h	+5 h	+1 h	+5 h
Ileum	215.1 ± 33.1	$107.2 \pm 7.2^\dagger$	155.3 ± 9.4	204.2 ± 23.9	$131.0 \pm 9.1^*$
Proximal colon	133.0 ± 17.9	$81.2 \pm 15.9^*$	130.8 ± 22.4	130.0 ± 20.5	$72.8 \pm 9.7^*$

Values (no. of intact mast cells/mm²) are means $\pm$ SE; $n = 6$ –8 rats. LPS, lipopolysaccharide. * $P < 0.05$, † $P < 0.01$ significantly different from corresponding control values.

Table 2. Kinetics of rhIL-1 β and rboTNF- α effects on abdominal response to gradual distension in awake rats

	3 h	6 h	9 h	12 h
Vehicle (0.3 ml/rat)	3.9 ± 1.2	4.2 ± 1.0	6.9 ± 1.5	7.2 ± 2.0
rhIL-1 β (10 μ g/kg ip)	$14.0 \pm 3.0^*$	$11.9 \pm 3.1^*$	$14.1 \pm 2.2^*$	9.6 ± 2.2
Vehicle (0.3 ml/rat)	3.7 ± 2.2	3.5 ± 2.6	4.8 ± 3.4	5.5 ± 2.5
rboTNF- α (150 μ g/kg ip)	12.3 ± 5.7	$19.0 \pm 2.0^\dagger$	$16.8 \pm 2.9^*$	7.0 ± 4.5

Values are means $\pm$ SE of the number of abdominal contractions during 5-min periods at the 0.4-ml volume of distension; $n = 6$ –8 rats. Recombinant human interleukin-1 β (rhIL-1 β) and recombinant bovine tumor necrosis factor- α (rboTNF- α) increased the number of abdominal contractions elicited at the 0.4-ml volume of distension from 3 to 9 h and from 6 to 9 h after injections, respectively. * $P < 0.05$, † $P < 0.001$ significantly different from corresponding vehicle values.

Involvement of IL-1 β in LPS-Induced Delayed Visceral Allodynia

After intraperitoneal injection of rhIL-1 β (10 μ g/kg), a significant ($P < 0.05$) increase of the number of abdominal contractions generated by the 0.4-ml volume of distension, compared with vehicle, was observed 3, 6, and 9 h after rhIL-1 β administration (Table 2). No significant increase of abdominal contractions was observed after intraperitoneal injection of saline for the same volume of distension, compared with baseline contractions, at any time of RD after saline. The tripeptide Lys-D-Pro-Thr (10 mg/kg ip) did not affect per se the abdominal response induced by RD at any volume of distension applied. However, when injected 30 min before LPS (1 mg/kg ip), the tripeptide significantly decreased ($P < 0.05$) the number of abdominal contractions at the distension volume of 0.4 ml (Fig. 4, A and B) 12 h after LPS (7.2 ± 2.0 vs. 15.8 ± 3.7 for LPS + vehicle control).

Involvement of TNF- α in LPS-Induced Delayed Visceral Allodynia

Similarly to rhIL-1 β , systemic administration of rboTNF- α (150 μ g/kg ip) regenerated rectal allodynia; intraperitoneal administration of rboTNF- α increased the number of abdominal contractions induced by RD at the 0.4-ml volume by 67% and 58% at 6 and 9 h after administration, respectively, compared with control animals treated with vehicle (Table 2). sTNFR (2 mg/kg ip) had no effect per se on basal rectal sensitiv-

Fig. 4. Effect of tripeptide Lys-D-Pro-Thr on LPS-induced increase of number of abdominal contractions in response to rectal distension at different volumes of distension (A) and at the lowest volume of distension (0.4 ml; B). Lys-D-Pro-Thr (10 mg/kg ip) significantly reduced the number of abdominal contractions at the 0.4-ml volume of distension 12 h after LPS administration. Values are means $\pm$ SE; $n = 5$ rats. **Significant difference ($P < 0.01$) from corresponding control values. †Significant difference ($P < 0.05$) from corresponding LPS + vehicle value.

ity, but, when injected before LPS, sTNFR significantly ($P < 0.05$) reduced the LPS-induced increase in the number of abdominal contractions observed at the 0.4-ml volume (Fig. 5, A and B) 12 h after LPS administration (4.7 ± 0.9 vs. 15.5 ± 2.1 contractions/5 min for LPS + vehicle control).

Involvement of Vagus Nerves in LPS-Induced Delayed Visceral Allodynia

CCK-8S significantly ($P < 0.05$) inhibited food intake in sham-vagotomized but not in vagotomized animals, thereby confirming the role of the vagus nerve in the CCK satiety test. Food intake was significantly decreased in CCK-injected sham-vagotomized rats compared with saline control sham-operated animals (1.8 ± 0.5 vs. 4.5 ± 0.4 g), whereas CCK-8S did not modify food intake in vagotomized animals compared

with corresponding saline-injected vagotomized animals (3.7 ± 0.7 vs. 4.4 ± 0.4 g).

After vehicle treatment, subdiaphragmatic truncal vagotomy did not affect the number of abdominal contractions compared with sham-vagotomized animals at any volume of RD (Fig. 6). In sham-vagotomized animals, LPS increased the number of abdominal contractions 12 h after its administration at the RD volume of 0.4 ml (14.7 ± 1.4 vs. 4.9 ± 2.3 abdominal contractions/5 min for vehicle control), similarly to what was observed in intact animals. Surprisingly, marked differences occurred between sham-vagotomized and vagotomized groups after LPS injection (Fig. 6): the number of abdominal contractions at the lowest volume of distension (0.4 ml) reached 23.9 ± 4.4 contractions/5 min in vagotomized animals, a value significantly higher than that observed in sham-vagotomized ani-

Fig. 5. Effect of peripheral administration of soluble tumor necrosis factor receptor (sTNFR) on LPS-induced increase of number of abdominal contractions in awake rats at different volumes of distension (A) and at the lowest volume of distension (0.4 ml; B). Note that sTNFR (2 mg/kg ip) significantly reduced the number of abdominal contractions elicited by LPS injection only at the volume of 0.4 ml 12 h after LPS. Values are means $\pm$ SE; $n = 6$ rats. *Significant difference ($P < 0.05$) from corresponding control values. ††Significant difference ($P < 0.01$) from LPS + vehicle-treated animals.

Fig. 6. Influence of vagotomy vs. sham operation on LPS-induced delayed (12 h) rectal hypersensitivity to distension. The response to LPS is amplified in vagotomized animals. Values are means $\pm$ SE; *n*, no. of rats. Significant difference from corresponding vehicle control value: **P* < 0.05, ***P* < 0.01. †Significant difference (*P* < 0.05) from corresponding sham + LPS value.

mals (14.7 ± 1.4 contractions/5 min for sham + LPS value).

DISCUSSION

The present experiments provide new insights regarding the effects of LPS on visceral sensitivity as previously investigated for somatic pain (43, 46). First, intraperitoneal injection of LPS triggers a delayed lowering (9–12 h) of the threshold of rectal distension-induced nociception in rats. Second, among the various proinflammatory mediators released in response to LPS, IL-1 β and TNF- α appear to have a critical role in the genesis of LPS-induced rectal allodynia. Third, the nociceptive response observed after LPS administration is attenuated by doxantrazole, a mast cell stabilizer, and histological studies confirmed gut mucosal mast cell degranulation after LPS injection. Fourth, subdiaphragmatic vagotomy, surprisingly, increases the magnitude of rectal allodynia induced by LPS. Together, these data lead to the conclusion that intraperitoneal LPS evokes a mast cell- and cytokine-dependent delayed rectal allodynia controlled by vagus nerves.

In the first part of the study, we demonstrated that intraperitoneal LPS decreases rectal pain threshold but does not modify the magnitude of response to noxious volumes of distension. These data suggest that LPS released during infection favors an abnormal visceral sensitivity to mechanical stimuli, corresponding to a lowered threshold of barosensitivity, without affecting the visceral pain intensity evoked by noxious stimulation. The same observations were described previously after inflammation of colorectal mucosa by

trinitrobenzene sulfonic acid in ethanol (29). The mechanisms evoking such abnormal visceral sensitivity are not yet fully understood. Such abnormal pain sensation, known as allodynia, could be related to a sensitization of low-threshold primary afferents, known to transmit nonpainful sensations in normal conditions and to be able to trigger postsynaptic nociceptive messages in inflammatory conditions. Such alterations have already been observed after nerve injury and cutaneous inflammation in somatic sensitivity (2, 12, 21). In the present study, the absence of change in the abdominal response for higher volumes may be related to the lack of sensitization of high-threshold afferents during the 12 h after LPS administration. Such a hypothesis might explain why LPS turns rectal perception into an abnormal one (allodynia) without modifying the pain response magnitude evoked by noxious stimuli.

We have also observed that intraperitoneal LPS enhances rectal sensitivity in two distinct periods, i.e., 3 h (early phase) and from 9 to 12 h (late phase) after its administration. We can attribute the early phase to a direct effect of endotoxin or pronociceptive mediators, such as PGE₂ released from macrophages, acting on primary afferent terminals and the late phase to the subsequent development of inflammation with an intense activation of primary afferent fibers and changes in spinal or central processing (25, 32). In agreement with such a hypothesis, most reports related to endotoxin-induced somatic hyperalgesia have measured a decrease in the latency to cutaneous nociceptive stimulus that occurred in the first hour after intraperitoneal injection of LPS (44, 46). In addition, a model of localized inflammatory hyperalgesia was recently developed in rats using intraplantar injection of endotoxin in the hind paw (15). In this study, Kanaan et al. (15) also observed a peak of hyperalgesia 9 h after endotoxin injection with complete recovery 24 h later, and they explained this delayed response by a similar time-related occurrence of a localized inflammatory reaction.

Different patterns of body temperature profile (monophasic fever, biphasic fever, and hypothermia/hyperthermia) have been described depending on the dose of LPS used (36). At a high dose (≥ 1 mg/kg), LPS triggers first a 1- to 3-h decrease of body temperature (hypothermia) followed by a long (6–24 h) period of fever (37, 47). In the present experiments, intraperitoneal administration of a high dose of LPS, i.e., 1 mg/kg, evokes a biphasic fever with two peaks of hyperthermia 2 and 5–6 h later, but we have never observed an initial hypothermia in the first hour after injection. Such a discrepancy may be related to LPS preparation or serotype, rat strain, or route of administration, as previously reported (11, 15).

In the third part of the study, we showed that LPS-induced delayed allodynia is attenuated by an IL-1 β receptor antagonist, the tripeptide Lys-D-Pro-Thr. This result agrees with previous observations in which LPS-induced somatic hyperalgesia was also abolished by intraperitoneal administration of the IL-1 receptor an-

tagonist (22) and by this tripeptide (39). Moreover, Kanaan et al. (15) also suggested the involvement of IL-1 β in the mediation of both endotoxin-induced thermal and mechanical hyperalgesia. Moreover, intraperitoneal IL-1 β reproduces the nociceptive response of LPS on the pain threshold to rectal distension with a time course of 3–9 h. Similarly, sTNFR, which acts as a functional TNF antagonist, reduces LPS-induced delayed allodynia (12 h), suggesting that TNF- α participates in the delayed decrease of rectal pain threshold after LPS. These results are also in agreement with data obtained for somatic pain. Indeed, the cutaneous hyperalgesic effect of LPS can be blocked by TNF- α binding protein, which acts as a functional TNF antagonist (42, 44). Intraperitoneal TNF- α administration can also reproduce LPS nociceptive response with a delayed maximal response appearing between 6 and 9 h. A long-lasting somatic hyperalgesia after intraplantar TNF- α administration has also been reported (4).

Changes in visceral sensitivity related to LPS could be secondary to the activation of chemosensitive nociceptors by inflammatory and/or proalgesic mediators (32). Several chemicals produced by local cells are capable of changing the sensitivity of nociceptors, including bradykinin, histamine, 5-hydroxytryptamine, neuropeptides such as substance P and calcitonin gene-related peptide, prostaglandins (5), and also cytokines (4, 7). LPS stimulates the expression of a large number of cytokines, particularly IL-1 β and TNF- α , that may act directly on receptors found on neurons or indirectly by stimulating the release of proalgesic substances that can act on neurons in a cascadelike manner (40). For example, IL-1, as well as LPS, stimulates the arachidonic acid cascade, resulting in prostanoid production and release (28) that sensitize primary afferent nociceptors and augment the excitability of sensory afferents (5). However, other mediators such as bradykinin can initiate both the cascade of cytokine release that mediates hyperalgesic response to endotoxins (9) and activation and sensitization of pain nociceptors (14). In contrast to these data, IL-1 can also induce hyperalgesia by acting directly on high-threshold mechanoreceptors, leading to a decrease in latency of neuronal discharges, to a lowering of the threshold, and to an increase of neuronal response to mechanical and thermal stimulation (10). In fact, these observations suggest a dual action of IL-1 β on different structures such as immune cells and the endings of terminal neurons, depending on the pathophysiological context. Concerning the potential involvement of TNF- α in the LPS cascade, several lines of evidence suggest that TNF- α can exert its effects through a cascade of cytokine release rather than by a direct stimulation of sensory afferent neurons. Indeed, TNF- α is the first cytokine released after LPS administration, and it reaches a maximal plasma concentration after 1 h (48). In fact, TNF- α may produce hyperalgesia by inducing the secondary release of IL-1 β , because its effect can be blocked by an IL-1 receptor antagonist (42). In carrageenan-evoked somatic hyperalgesia, the same mech-

anism has been reported: bradykinin induces the release of TNF- α , which in turn stimulates the release of other hyperalgesic cytokines (IL-1 β , IL-6, and IL-8) responsible for the generation of cyclooxygenase products and sympathomimetic amines (8). In consequence, these two cytokines seem to play a role in LPS-induced visceral hypersensitivity at different levels and in a cascade.

Doxantrazole, a mast cell stabilizer, when injected before LPS, prevents both the early (3 h) and the late (12 h) phase of LPS-induced rectal allodynia. Consequently, mast cell activation appears to be involved in the cascade of reactions leading to the nociceptive response related to LPS administration. Concerning the early phase (3 h), a previous report showed that LPS can directly degranulate mast cells with production of cytokines without substantial release of preformed mediators by exocytosis (20). Moreover, other previous studies report the importance of various immune cells, particularly resident macrophages, from the liver in the production of somatic hyperalgesia appearing early within 1 h after intraperitoneal LPS administration (43, 44). Together, these observations permit us to suggest that the early phase could be linked to the activation of immune cells, and particularly mast cells, present in sites other than the gut wall because this early nociceptive response is abolished by previous treatment with a mast cell stabilizer, doxantrazole, and because no immediate mucosal mast cell degranulation is observed histologically in the gut. Concerning the late phase (12 h), we suggest that this phase is related to the development of an inflammatory reaction triggered by LPS and involving a delayed gut mucosal mast cell degranulation. Indeed, our histological study shows a decrease in mucosal mast cell number occurring 5 h after LPS administration, and this phase is abolished by doxantrazole. Moreover, this hypothesis is in agreement with a previous study showing that BrX-537A, a potent gut mucosal mast cell degranulator (30), promotes only a delayed rectal allodynia observed from 6 to 12 h after its administration (3). Furthermore, we have shown here that BrX-537A triggers an immediate (<1 h) mucosal mast cell degranulation in the gastrointestinal tract. Consequently, we can hypothesize that the late phase of rectal allodynia, consecutive to intraperitoneal LPS administration, is linked to resident mast cell degranulation localized in the intestinal tract.

Vagus nerve serves as an informational highway for inflammatory signals from the periphery, and bilateral truncal vagotomy abolishes a wide range of behavioral and neural effects of peripheral administration of LPS. A part of the "illness" signals elicited by intraperitoneal cytokines or LPS is relayed directly to brain primarily by the vagus nerve, which activates a centrifugal pain facilitatory pathway. Indeed, proinflammatory cytokines (IL-1 β and TNF- α) produce somatic hyperalgesia by activating vagal afferents (42, 44). In contrast, our data demonstrate that total subdiaphragmatic vagotomy amplifies LPS-induced rectal allodynia and thus that the vagus nerve has a protective effect

against LPS-induced visceral allodynia. Chemical, electrical, or physiological activation of cardiopulmonary, diaphragmatic, or subdiaphragmatic vagal afferents can result in either facilitation or inhibition of nociception in some species, depending on the intensity of stimulation (for review, see Ref. 31). For example, high-intensity stimulation of vagal afferents activates spinal descending inhibitory systems, whereas low intensity stimulates pain facilitatory circuits (34). Therefore, it can be postulated that, according to the intensity of the stimulus applied in our study, vagal afferent fibers can participate in a feedback loop directly controlling chemical nociceptive inputs from the periphery by activating descending antinociceptive pathways. However, there is substantial evidence that sensory neurons have a protective role against injury to the gut (6, 33). Indeed, worsening of inflammation has been observed in acute colitis models after perivagal capsaicin pretreatment, suggesting a direct protective function of vagal afferents on colonic mucosa against inflammation (23). Thus we can hypothesize that similar processes may underlie the protective action of vagus nerve against local inflammatory reactions resulting from LPS administration. However, it can also be hypothesized that the ability of total subdiaphragmatic vagotomy to reduce LPS hyperalgesia, in some studies, or to amplify the same response, as seen in our data, may be due to differences in the dose of LPS applied. Indeed, the dose of 1 mg/kg is very high compared with doses used in somatic models (16, 43), inducing, in particular, fever and behavioral or locomotive disturbances. In consequence, the vagus nerve appears to possess a powerful role in mediating peripheral immune signals to the brain, but its role must be different according to the nature and/or the amplitude of the aggression and the pathophysiological context developed after LPS infection. It is clear, however, that multiple circuits mediating pain responses exist and are activated under different circumstances.

In summary, the present study indicates for the first time that peripherally injected LPS lowers the visceral pain threshold to rectal distension, this allodynia being observed between 9 and 12 h, and that this effect is mediated by two cytokines, IL-1 β and TNF- α , and involves mast cell degranulation. Because intraperitoneal administration of LPS is associated with inflammatory reactions in the gut, this result adds some insights into possible mechanisms by which immune reactions of the brain-gut axis may participate in the genesis of visceral allodynia.

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