A role for cyclooxygenase-2 in lipopolysaccharide-induced anorexia in rats

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Lugarini, F., B. J. Hrupka, G. J. Schwartz, C. R. Plata-Salamán, and W. Langhans. A role for cyclooxygenase-2 in lipopolysaccharide-induced anorexia in rats. Am J Physiol Regul Integr Comp Physiol 283: R862–R868, 2002.—Because nonselective cyclooxygenase (COX) inhibition attenuated anorexia after lipopolysaccharide (LPS) administration, we tested the ability of resveratrol (2.5, 10, and 40 mg/kg) and NS-398 (2.5, 10, and 40 mg/kg), selective inhibitors of the two COX isoforms COX-1 and -2, respectively, to attenuate LPS (100 μg/kg ip)-induced anorexia.

NS-398 (10 and 40 mg/kg) administered with LPS at lights out attenuated LPS-induced anorexia, whereas resveratrol at all doses tested did not. Because prostaglandin (PG) E2 is considered the major metabolite synthesized by COX, we measured plasma and cerebrospinal fluid (CSF) PG E2 levels after LPS administration. LPS induced a time-dependent increase of PG E2 in CSF but not in plasma. NS-398 (5, 10, and 40 mg/kg) blocked the LPS-induced increase in CSF PG E2, whereas resveratrol (10 mg/kg) did not. These results support a role of COX-2 in mediating the anorectic response to peripheral LPS and point at PG E2 as a potential neuromodulator involved in this response.

NS-398; resveratrol; prostaglandin E2; food intake; fever

LIPOPOLYSACCHARIDE (LPS) and proinflammatory cytokines [e.g., interleukin (IL)-1β, IL-6, and tumor necrosis factor-α] induce anorexia and fever (32), two phenomena partly independent of each other. Brain areas involved in feeding and body temperature regulation are activated (5, 40) after peripheral administration of LPS and cytokines. This can be accomplished through neural and/or humoral communications with the central nervous system (CNS). Vagal afferents have been implicated in some of the behavioral effects induced by peripheral LPS or cytokines (3, 16). In our hands, however, subdiaphragmatic vagal deafferentation, alone or in combination with celiac-superior mesenteric ganglionectomy, did not attenuate the anorectic response to peripheral LPS, muramyl dipeptide, and IL-1β (33). An alternative route of communication between the periphery and the CNS involves cytokine and LPS receptors on the surface of the cerebral endothelial cells of the brain-blood barrier (1, 39) and subsequent mediators such as prostaglandins (PGs) (6). PGs are synthesized by cyclooxygenase (COX), an enzyme that exists in two different isoforms. COX-1 is constitutively expressed in many tissues, mainly outside the brain, and its levels are relatively insensitive to inflammatory stimulation. COX-1 is scarcely expressed in capillary endothelial and perivascular glial cells (13). COX-2, on the other hand, is constitutively expressed at low levels in neurons of the cortex, hippocampus, and amygdala, but not in the cells of the cerebral vasculature (34). COX-2 is strongly induced in brain vasculature, however, by LPS and IL-1β (12, 22, 34). LPS or cytokines (31) transiently enhance COX-2 mRNA and protein levels via activation of nuclear factor-κB (2, 23).

In our hands, nonselective pharmacological inhibition of COX by administration of indomethacin or paracetamol attenuated the pyretic and hypophagic effects of peripheral LPS and IL-1β (26, 27). Selective inhibition of COX-2 blocks LPS-induced fever (7). Use of selective inhibitors for COX-1 and -2 could not establish a specific role for either of the two COX isoforms as mediators of the anorectic effect of peripheral LPS and cytokines in mice (10). In this study, drugs were administered during the light period, when mice are not particularly active, and intake of a milk meal was measured only for 30 min. A recent study from the same laboratory (37) tried to exclude a role for COX-1 in LPS- and IL-1β-induced hypophagic response using COX-1 and COX-2 knockout mice. In this study, however, LPS was tested only in COX-1 knockout mice, in which it reduced milk intake as in wild-type controls. It should be noted in this context that interpretation of negative results in knockout preparations is limited because developmental compensation may occur.

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To further investigate the role of COX-1 and COX-2 in the anorexia induced by peripheral LPS, we tested the effects of different doses of selective inhibitors of COX-1 (resveratrol) and COX-2 (NS-398) on the feeding suppressive effect of an estimated pathophysiological dose of LPS in rats fed chow ad libitum. Because PGE₂ is the major product of COX, we analyzed PGE₂ levels in plasma and cerebrospinal fluid (CSF) after peripheral LPS administration, and we determined the effect of COX-1 and COX-2 inhibition on CSF PGE₂ levels.

**METHODS**

**Animals and housing conditions.** Male Sprague-Dawley rats were individually housed in stainless steel hanging wire cages with wire-mesh bottoms. Founder rats from Charles River Germany were maintained as a breeding colony under specific pathogen-free conditions in our animal facility. Animal rooms were maintained at 22 ± 0.5°C on a 12:12-h light-dark cycle. Standard powdered laboratory chow (890, Nafag, Grossau, Switzerland) and water were available ad libitum. Before experiments, rats were adapted to maintenance conditions for at least 10 days and were handled daily.

All procedures and protocols were approved by the canton of Zurich’s Animal Use and Care Committee.

**Test procedures.** Before each experiment, rats were ranked according to the food intake recorded the previous 24 h (baseline food intake) in the feeding experiments (experiments 1 and 2) or according to body weight in the PGE₂ sampling experiments and were randomly assigned within blocks to treatment groups. In feeding experiments, rats were food deprived during the light phase before the experiment, and the preweighed food cups were returned to the cages at lights out, after drug administration. Unless otherwise noted, all injections were given intraperitoneally at lights out. Drug solutions were prepared freshly before administration. LPS (from Escherichia coli, serotype 0111:B4, no. L-2630, Sigma) was dissolved in isotonic pyrogen-free saline and injected at a dose of 100 μg/kg throughout all the experiments. This dose was selected based on previous studies (24, 25). The COX-2 inhibitor NS-398 (RBI, no. N-194) was either suspended in gum tragacanth (no. G-1128, Sigma) and administered by oral gavage or dissolved in DMSO (no. D-8779, Sigma)/saline and injected intraperitoneally. Resveratrol (RBI, no. R-132) was dissolved in DMSO/saline and injected intraperitoneally. Control treatments consisted of the equivalent volume of appropriate vehicle.

**Food intake measurements.** Food intake was always recorded 3, 6, 9, 12, and 24 h after drug/LPS administration by manually weighing the food cups (+0.1 g) and correcting for spillage that was collected on papers placed under the animal cages. During the dark phase, a red light was used to illuminate the room.

**Temperature measurements.** Rectal temperature was measured by inserting a thermoprobe (Indulah, Buchs, Switzerland) 3 cm into the rectum (+0.1°C). Rats were gently held during the temperature measurement. This procedure was performed a few times on different days before the experiment to minimize the possible stress-induced temperature changes (4).

**Blood and CSF sampling.** Blood and CSF sampling were both performed as terminal procedures. Blood was collected from rats anesthetized by intraperitoneal injection (1 ml/kg) of a mixture of 80 mg/kg ketamine HCl (Narketan 10, Chas-sot, Bern, Switzerland) and 4 mg/kg xylazine (Rompun, Bayer, Leverkusen, Germany). A cutaneous incision on the midline of the upper abdomen was made that extended to the chest cavity, exposing the heart. Blood (5–8 ml) was aspirated by heart puncture from the right ventricle with a needle and a syringe. Blood was then placed in polypropylene test tubes treated with 50 μl of solution containing 9 mg EDTA, 0.057 mg sodium carbonate, and 50 μg indomethacin (the latter to inhibit the activity of COX enzyme, i.e., to block the synthesis of PGE₂) and stored on ice for not more than 30 min before centrifugation (1,600 g at 8°C for 12 min). Plasma samples from each rat were then divided in aliquots of 500 μl and stored at −20°C until determination of PGE₂ concentration.

CSF was collected from rats anesthetized as described above. Rats were placed in a stereotaxic apparatus, and the neck was flexed so that a 29-gauge needle could be lowered between the base of the skull and the first cervical vertebra, in the cisterna magna. Needle placement was verified by drawing a small amount of CSF into the injection needle and observing the clear CSF in the Tygon tubing that connected the needle to the 50-μl syringe (Hamilton). CSF (20–30 μl) was collected in polypropylene test tubes that were put immediately on dry ice and then stored at −80°C until determination of PGE₂ concentration. After dilution (1:10) of the CSF samples, PGE₂ was measured with an ELISA (PGE₂ High Sensitivity Immunoassay Kit, R&D System) following the procedures detailed in the instructions. The sensitivity of this ELISA kit was <8.25 pg/ml. The intra-assay and inter-assay coefficients of variation were 2.6 and 2.2%, respectively.

**Experiment 1, trial 1: dose-response curve of NS-398 in LPS-treated rats.** Because indomethacin, a nonselective inhibitor of COX activity, attenuated LPS-induced anorexia, 28 rats were used to test the ability of NS-398, a selective COX-2 inhibitor, to reverse LPS-induced anorexia. One hour before lights out, rats (302 ± 4 g body wt, mean ± SE) received either 0, 2.5, 10, or 40 mg/kg NS-398 dissolved in 1% gum tragacanth and administered by oral gavage at a volume of 2 ml/kg. At lights out, all rats were injected with LPS. Food intake was recorded as described under METHODS.

**Experiment 1, trial 2: 2 × 2 factorial arrangement with NS-398 (10 mg/kg) and LPS.** Because the 10 mg/kg dose of NS-398 attenuated LPS-induced anorexia in the previous trial, we examined the effect of NS-398 on the feeding-suppressive effect of LPS in a 2 × 2 factorial design using 28 animals (331 ± 5 g body wt, mean ± SE). At lights out, half of the rats received LPS injections while the other half received saline. Half of the rats in each group (LPS vs. saline) received intraperitoneal injections of either NS-398 (10 mg/kg) or DMSO diluted with saline (50:50 vol/vol). Food intake was recorded as described above.

**Experiment 2, trial 1: dose-response curve of resveratrol in LPS-treated rats.** To further investigate the specific role of the two COX isomers on food intake of LPS-treated rats, 28 rats were used in this experiment to test the effect of resveratrol, a selective inhibitor of COX-1, on LPS-induced anorexia. At lights out, all rats (294 ± 3 g body wt, mean ± SE) received injections of LPS and of either 0, 2.5, 10, or 40 mg/kg resveratrol dissolved in DMSO and saline (50:50 vol/vol). Food intake was recorded as described above.

**Experiment 2, trial 2: 2 × 2 factorial arrangement with resveratrol (10 mg/kg) and LPS.** Twenty-eight rats (322 ± 7 g body wt, mean ± SE) were used in this experiment to investigate the effect of resveratrol on the feeding-suppressive effect of LPS. At lights out, half the rats received LPS injections (100 μg/kg ip) while the other half received saline. Within each group (LPS vs. saline), half the rats received
intrapерitoneal injections of either resveratrol (10 mg/kg) or DMSO, diluted with saline (50:50 vol/vol). Food intake was recorded as described above.

Experiment 3: antipyretic effect of NS-398 (5 mg/kg) on LPS-treated rats. Because LPS is reported to induce fever after peripheral administration, and because activation of COX-2 is believed to be involved in the development of LPS-induced fever, we tested the antipyretic effect of NS-398 (5 mg/kg) in LPS-treated rats. This dose did not attenuate the LPS-induced anorexia in a previous experiment of ours in which we employed a 2 × 2 factorial design of NS-398 and LPS (data not shown). Because the circadian thermoregulatory rhythm in rats consists of a stable daytime phase with a body temperature around 37.3°C, we chose to induce fever in the middle of the light phase, when a rise in body temperature is detectable. The 12:12-h light-dark cycle in the animal room was set with lights out at 1400. From 0600 to 0800, the rats’ basal temperature (n = 10, 447 ± 11 g body wt, mean ± SE) was determined four times, at 30-min intervals. The mean basal temperature for each experimental group is shown in Fig. 3. In the middle of the light phase (0800), half the rats received NS-398 by oral gavage suspended in 1% gum tragacanth and administered at a volume of 2 ml/kg, while the others received the same amount of vehicle. One hour later (0900), all rats received an injection of LPS. Rectal temperature was then measured every 30 min over a period of 6 h after the LPS injections.

Experiment 4, trial 1: time course of PGE2 levels in plasma of LPS-treated rats. To establish the concentration of PGE2 in the systemic circulation after LPS treatment, and to relate this to the LPS-induced food intake reduction, 35 rats (386 ± 6 g body wt, mean ± SE) were assigned to experimental groups, which involved blood collection at 0 (control), 1, 2, 3, and 4 h after LPS administration. The experiment was conducted on 2 consecutive experimental days with two to three rats per group treated each day. Within each group, LPS injections were staggered at lights out (1400) to allow 5 min for blood collection per animal.

Experiment 4, trial 2: time course of PGE2 levels in CSF of LPS-treated rats. Thirty-five rats (278 ± 3 g body wt, mean ± SE) were used to establish the concentration of PGE2 in the CSF after LPS treatment and to relate this to the LPS-induced food intake reduction. At lights out (1400), all rats were injected with LPS, and CSF samples were collected at 0 (control), 1, 2, 3, and 4 h after the injections in the same manner described in experiment 4, trial 1.

Experiment 4, trial 3: effect of COX inhibitors on CSF PGE2 levels at 4 h after LPS administration. Because we detected in the previous experiment a maximal raise of PGE2 in CSF 4 h after LPS administration, this time point was chosen to compare the effect of LPS and different doses of NS-398 and resveratrol on CSF PGE2 concentrations. Forty-nine rats (298 ± 2 g body wt, mean ± SE) were assigned to seven experimental groups. Rats of each group were staggered in 7 consecutive experimental days to test 7 rats/day, one from each experimental group. At lights out (1200), rats received an intraperitoneal injection of either 1) saline, 2) LPS alone, 3–5) LPS + 5, 10, and 40 mg/kg of NS-398, respectively, 6) LPS + resveratrol (10 mg/kg), or 7) DMSO (vehicle), and the food cups were weighed. Four hours later (1600), food cups were weighed again to calculate 4-h food intake. Rats were anesthetized, and CSF samples were collected and treated as described under METHODS.

Statistical analysis. Results from dose-response trials were analyzed using general linear model (GLM) procedures appropriate for a one-way ANOVA with blocking (SAS, SAS Institute, Carey, NC, release 6.12). When an ANOVA revealed overall significant differences, treatment means were compared using Duncan’s multiple range test. Results from LPS × drug interaction trials were analyzed using GLM procedures appropriate for a 2 × 2 factorial arrangement of LPS and drug with replicates. Replicates consisted of rats with similar body weights or similar baseline food intake. Results are expressed as means ± SE.

RESULTS

Experiment 1, trial 1: dose-response curve of NS-398 in LPS-treated rats. NS-398 (10 and 40 mg/kg) attenuated the hypophagic effect of LPS (Fig. 1A). For the 40-mg/kg dose, this effect was significant at 3, 6, 9, and 12 h after administration (all P < 0.05). Although rats receiving 10 mg/kg NS-398 ate consistently more than LPS-treated control rats, the Duncan’s test was significant only at 3 h.

Experiment 1, trial 2: 2 × 2 factorial arrangement with NS-398 (10 mg/kg) and LPS. As expected, LPS reduced food intake significantly (P < 0.0001), and this effect was attenuated by NS-398 administration (LPS × NS-398 interaction: P < 0.02, Fig. 1B). NS-398
administration did not alter food intake in rats that were not treated with LPS.

Experiment 2, trial 1: dose-response curve of resveratrol in LPS-treated rats. None of the doses of resveratrol administrated increased food intake of LPS-treated rats at any of the time points tested (all $P > 0.1$, Fig. 2A). Rats treated with 10 mg/kg resveratrol ate slightly more than controls (2–4.5 g, not significant); therefore, this dose was tested in experiment 2, trial 2.

Experiment 2, trial 2: 2 × 2 factorial arrangement with resveratrol (10 mg/kg) and LPS. LPS reduced food intake significantly ($P < 0.0007$, Fig. 2B). Unlike in experiment 2, trial 1, where resveratrol (10 mg/kg) slightly enhanced feeding, here resveratrol suppressed food intake at 6, 9, 12, and 24 h in LPS-treated rats (LPS × resveratrol interaction, $P < 0.02$), whereas it did not affect control rats’ food intake.

Experiment 3: antipyretic effect of NS-398 (5 mg/kg) on LPS-treated rats. LPS treatment induced a rise in the rectal temperature ($P < 0.01$, Fig. 3). Pretreatment with 5 mg/kg NS-398 blocked the LPS-induced rise in temperature at 1 and 2 h after LPS injection and significantly inhibited it from 3 to 6 h.

Experiment 4, trials 1 and 2: time course of PGE$_2$ levels in plasma and CSF of LPS-treated rats. Basal plasma and CSF PGE$_2$ levels in rats were 343 ± 106 and 324 ± 112 pg/ml, respectively (values at 0 h, Fig. 4). Intraperitoneal injection of LPS (100 µg/kg) at lights out resulted in a significant ($P < 0.02$) and steady increase in CSF PGE$_2$ levels for 0–4 h after LPS administration. Plasma PGE$_2$ levels did not increase after LPS administration and were not significantly different from the 0-h values at any time point tested.

Experiment 4, trial 3: effect of COX inhibitors on CSF PGE$_2$ levels at 4 h after LPS administration. LPS administration resulted in a sixfold increase of the concentration of PGE$_2$ at 4 h after the injections (Fig. 5, LPS/vehicle vs. saline/vehicle). The CSF PGE$_2$ level of rats treated with LPS and NS-398 (5, 10, and 40 mg/kg) was similar to control regardless of NS-398 dose. Resveratrol administration in LPS-treated rats...
response is not secondary to fever (28). Our data sug-
et that fever is more sensitive to inhibition of the
COX-2 pathway than anorexia. The inconsistent atten-
uation of LPS anorexia with the 5 mg/kg dose of NS-
398 could indicate that 5 mg/kg is about the threshold
dose of NS-398 for the attenuation of the LPS-induced
anorexia.

In control rats, resveratrol did not alter food intake,
and we found no consistent effect of resveratrol in
LPS-treated rats. Resveratrol either attenuated or en-
hanced LPS-induced anorexia depending on the dose
and/or the trial, but most of the doses tested actually
enhanced the LPS-induced decrease of food intake.
This may reflect a COX-1-related protective role of PGs
in response to LPS. A potentiation of LPS anorexia by
COX-1 inhibition was recently found in mice (19). Un-
der certain conditions, such as when exogenous IL-1β
reduces milk intake in mice, COX-1 may be important
in mediating the early (first 30 min) period of anorexia
(11). However, our data scarcely suggest a role of
COX-1 in LPS-induced anorexia.

While plasma PGE2 levels were unchanged after
LPS injection, CSF PGE2 levels began to rise by 1–2 h
and were increased sixfold compared with controls by
4 h (see Figs. 4 and 5). Clearly, there is an association
between the increase in CSF PGE2 levels and the onset
of anorexia and no association between plasma PGE2
and anorexia. The increase in CSF PGE2 probably
reflects the induction of COX-2 in the brain capillar-
y endothelial cells where LPS or cytokines transiently
enhance COX-2 mRNA and protein levels (31). COX-2
mRNA expression in brain capillary cells is enhanced
as early as 1 h after peripheral LPS administration
(100 µg/kg ip) (6), which is compatible with the devel-
opment of anorexia and other LPS-induced effects,
such as fever and activation of the hypothalamic-pitu-
ary-adrenal axis (35), which are also reported to be
mediated by COX-2 and CNS PGs.

Administration of 5, 10, and 40 mg/kg NS-398 all
completely blocked the LPS-induced increase in CSF
PGE2. At first glance this appears to be at odds with
the seemingly dose-related effect of NS-398 on LPS-
nduced suppression of food intake (2.5 mg/kg clearly
failed to attenuate LPS-induced anorexia, whereas 10
and 40 mg/kg did). Several non-mutually exclusive
explanations for this discrepancy are possible: the lack
of a dose-dependent effect of NS-398 on CSF PGE2
levels could be due to the fact that COX-2 activity is
already maximally inhibited with the 5-mg/kg dose,
and although PGE2 may be the major mediator, other
arachidonic acid metabolites synthesized by COX-2
could contribute to the feeding-suppressive effect of
LPS. After systemic LPS administration, plasma
6-keto-PGF1 and thromboxane B2 have been shown to
be increased (15). Substances other than arachidonic
acid metabolites, for instance cytokines, such as IL-6
(8), not measured in this study and linked to down-
stream mediators other than arachidonic acid deriva-
tives, may also be involved. It should also be considered
that CSF PGE2 levels may not necessarily reflect the
PGE2 concentration at the neuronal site of action.
Finally, and as mentioned above, the 5-mg/kg NS-398
did not block the LPS-induced increase in CSF PGE2
level (Fig. 5, LPS/resveratrol vs. LPS/vehicle).

**DISCUSSION**

We provide evidence for a role of COX-2 in mediating
peripherally administered LPS-induced anorexia in
rats. Pharmacological inhibition of COX-2 by systemic
administration of NS-398 (10 mg/kg or more) attenu-
ated LPS-induced anorexia. In contrast, inhibition of
COX-1 did not attenuate LPS-induced anorexia or prevent the LPS-induced
increase in CSF PGE2. These results extend previous
findings in which nonselective COX inhibitors attenu-
ated intraperitoneal LPS-induced anorexia in rats (26),
mice (38), chickens (20), and pigs (21). Our results are
consistent with a recent study in which either pharma-
cological inhibition or genetic ablation of COX-2 in
mice also attenuated the body weight loss and anorec-
tic response of intraperitoneal LPS (19). Here we extend these findings by providing evidence
for a role of central PGE2 in LPS-induced
anorexia.

Administration of 5 mg/kg NS-398 blocked LPS-in-
duced fever but did not consistently attenuate LPS-
induced anorexia. In fact, whereas 5 mg/kg NS-398
failed to attenuate the feeding-suppressive effect of 100
µg/kg ip in LPS in a previous experiment of ours employ-
ing a 2 × 2 factorial experimental design (6-h food
intake: 9.0 ± 0.6 g (controls) vs. 5.2 ± 1.2 g (LPS +
vehicle) vs. 5.3 ± 0.7 g (LPS + NS-398), data not
shown), the same dose attenuated the LPS-induced suppression of food intake in experiment 4, trial 3,
when food cups where weighed before CSF sampling
(4-h food intake: 6.0 ± 0.9 g (controls) vs. 3.2 ± 0.5 g
(LPS + vehicle) vs. 4.7 ± 0.5 g (LPS + NS-398), data
not shown). Although fever and anorexia are both in-
duced during bacterial infection and are related to
COX-2 activation, they are at least partly dissociable
events, and anorexia associated with the acute-phase
response is not secondary to fever (28). Our data sug-

![Graph](http://ajpregu.physiology.org/)

**Fig. 5.** Effect of NS-398 (5, 10, and 40 mg/kg) and resveratrol (10
mg/kg) on the LPS (100 µg/kg)-induced increase in CSF PGE2
concentration at 4 h after injections. Data are means ± SE of 7 rats/
treatment. The LPS-induced increase of CSF PGE2 was blocked by
all doses of NS-398 but not by resveratrol. *Different from saline/
vehicle (P < 0.05).
dose appears to be the threshold dose for an inhibition of LPS anorexia, and in this particular experiment this dose in fact inhibited LPS anorexia (see above).

Even 10 mg/kg of resveratrol did not decrease the CSF PGE$_2$ levels in LPS-treated rats. Although resveratrol is thought to be a specific inhibitor of COX-1 (18), at high concentrations it has been reported to reduce basal COX-2 activity in vitro (36). If resveratrol is nonspecific at high doses, it does not appear to be able to block inducible COX-2 activity after LPS; otherwise one would likely see an effect on food intake similar to that displayed by NS-398. Likewise, even if our high NS-398 doses (10 and 40 mg/kg) also inhibited COX-1, the lack of an effect of resveratrol on feeding or CSF PGE$_2$ levels would suggest that the role of any nonselective inhibition of COX-1 is minimal.

All in all, the present results are in line with the hypothesis that the endothelial and perivascular cells of the brain vasculature act as an interface between blood and brain by producing secondary mediators, such as PGs, which in turn act on neurons involved in feeding. Four subtypes of transmembrane receptors (EP1–EP4) for PGE$_2$ and/or other prostanoid receptors are expressed on neurons that project to the paraventricular nucleus of the hypothalamus, a major integrative center for the control of food intake and energy balance (14, 41). Nakamura et al. (29) have identified the PGE$_2$ receptor EP3 on serotonergic neuronal cell bodies in the raphe nucleus. Serotonin has been shown to be involved in LPS-induced anorexia in rats (17), and it is abundant in neurons originating from the midbrain dorsal raphe nucleus and projecting to the hypothalamus, including the paraventricular nucleus. A clear colocalization of c-fos and EP4 mRNA in the paraventricular nucleus and other hypothalamic nuclei has been found after LPS administration in rats (30), and inhibition of PG synthesis by administration of ketorolac abolished the IL-1β-induced c-fos expression and increase of EP4 mRNA expression in the paraventricular nucleus (42). Taken together, these data suggest a role of EP4 receptors in LPS- and cytokine-induced phenomena, such as fever and anorexia. A role for glucagon-like peptide-1 in mediating fever and anorexia induced by peripheral LPS has also been proposed (9). Thus various monoaminergic and peptidergic mechanisms might be involved in the CNS propagation of the feeding-suppressive effect of peripheral LPS, and it remains to be investigated whether these serotonergic and peptidergic mechanisms act in parallel or in series with the mediating function of COX-2 suggested here.

The evidence presented here supports the idea that COX-2 induced in the brain endothelial and perivascular cells is involved in anorexia elicited by peripheral LPS. It is important, however, to learn how LPS-induced COX-2 expression in the brain blood vessels relates to other parallel and/or downstream mechanisms for anorexia induction.

An intraperitoneal dose of 100 μg LPS may be pathophysiologically relevant because it mimics most of the clinical features of systemic gram-negative bacterial infection (24). Our results therefore point to COX-2 and its metabolites, such as PGE$_2$, as important players in the anorexia during bacterial infections and may provide some indications about possible strategies for therapeutic intervention. Eicosanoid inhibitors are widely used in clinical medicine because of their effective antifebrile and anti-inflammatory activities. Further studies are necessary to determine whether COX-2 inhibition can be beneficial in attenuating the anorexia associated with acute and chronic pathophysiological processes in humans.

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