COX-2 inhibition attenuates anorexia during systemic inflammation without impairing cytokine production

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Johnson, Paulette M., Sherri K. Vogt, Mary W. Burney, and Louis J. Muglia. COX-2 inhibition attenuates anorexia during systemic inflammation without impairing cytokine production. Am J Physiol Endocrinol Metab 282: E650–E656, 2002; 10.1152/ajpendo.00388.2001.—Anorexia and weight loss are frequent complications of acute and chronic infections and result from induction of cytokines, prostaglandins, and other inflammatory mediators that are critical for pathogen elimination. Selective attenuation of the hypophagic response to infection and maintenance of the production of factors essential for infection control would be a useful addition to antimicrobial therapy in the treatment of human disease. Here, we evaluate the relative contribution of cyclooxygenase (COX)-1 and COX-2-derived prostaglandins to anorexia and weight loss precipitated by systemic immune activation by lipopolysaccharide (LPS). Using COX isoform-selective pharmacological inhibitors and gene knockout mice, we found that COX-2 inhibition during LPS-induced inflammation results in preserved food intake and maintenance of body weight, whereas COX-1 inhibition results in augmented and prolonged weight loss. Regulation of neuropeptide Y, corticotropin-releasing hormone, leptin, and interleukin-6 does not change as a function of COX-2 inhibition after LPS administration. Our data implicate COX-2 inhibition as a therapeutic target to maintain nutritional status while still allowing a normal cytokine response during infection.

nonsteroidal anti-inflammatory drugs; knockout mice; appetite; cyclooxygenase-2

ACTIVATION OF THE IMMUNE SYSTEM during acute bacterial or viral infections results in generation of cytokines and other inflammatory mediators that are essential for infection control. These inflammatory mediators result in mononuclear cell chemotaxis and activation and local changes in blood flow and vascular permeability that facilitate pathogen eradication. In addition to these beneficial actions, factors induced during infection or other systemic inflammation also exhibit detrimental actions. One important consequence of inflammation is anorexia, accompanied by acute weight loss (17, 19, 37). With severe or prolonged infection or inflammation, these changes in nutrition can adversely affect resolution of infection, wound healing, and growth. The ability to selectively inhibit the anorectic response while preserving the actions of inflammatory mediators necessary for pathogen elimination would be a very useful addition to the antimicrobial therapy of infection.

Lipopolysaccharide (LPS), a major component of the outer cell wall of gram-negative bacteria, has been extensively utilized as a model for acute sepsis. Similar to bacterial infection, LPS administration results in fever, robust cytokine production, and anorexia in rodents (20, 30). Inhibition of cytokine production or action after LPS, specifically tumor necrosis factor (TNF)-α and interleukin (IL)-1β, attenuates LPS-induced anorexia (29, 30). However, inhibition of these proximal mediators of the inflammatory cascade would be expected to compromise survival in the setting of a live, replicating pathogen as suggested by the increased susceptibility of mice with genetic deficiency of TNF receptor 1 (31), IL-6 (14), and interferon-γ (6) to infection with bacterial agents such as Listeria monocytogenes.

Inhibition of prostaglandin production has also been shown to attenuate the anorectic response to LPS or IL-1β (18, 33), but the mechanism by which this occurs and the consequences for generation of a protective inflammatory response remain unclear. The first committed step in prostaglandin synthesis, catalyzed by prostaglandin H synthases, or cyclooxygenases, has served as an important therapeutic target for treatment of inflammatory disease (36). Recently developed nonsteroidal anti-inflammatory drugs (NSAIDs) are capable of selectively inhibiting the function of one of the two cyclooxygenase (COX) isoforms, COX-1 or COX-2 (21, 22, 36). Improved understanding of the physiological roles of these COX isoforms will allow novel applications of COX-selective drugs, with fewer adverse sequelae, such as gastritis and nephrotoxicity, than nonselective inhibitors.

In this study, we test the hypothesis that COX-selective inhibition will be efficacious in attenuating the anorectic response accompanying immune system activation without altering cytokine or adrenal responses. Using COX-1- and COX-2-selective pharmacological agents and knockout mice, we will determine...
the relative contribution of each isoform to inflammation-induced anorexia.

MATERIALS AND METHODS

Animal housing. Wild-type (WT), COX-1-deficient knock-out (KO) (16), and COX-2 KO (26) mice were housed on a 12:12-h light-dark cycle with ad libitum access to rodent chow unless otherwise indicated. For pharmacological inhibitor studies, male C3H/HeN mice (Harlan Sprague Dawley, Indianapolis, IN) 10–12 wk of age were evaluated (11, 34). Male COX-1 and COX-2 KO mice, evaluated at 8–14 wk of age, were of an outbred C57BL/6 × 129 background maintained by KO male × heterozygote female matings for the COX-1 KO allele and heterozygote × heterozygote matings for the COX-2 KO allele. Control mice designated “WT” for the COX KO experiments were WT and heterozygous littersmates arising from the COX-2 heterozygous matings. All mouse protocols were in accordance with National Institutes of Health (NIH) guidelines and approved by the Animal Care and Use Committee of Washington University School of Medicine.

LPS-induced anorexia. C3H/HeN male mice were singly housed and acclimated for ≥2 days to cages with grid bottoms before treatments. Body weight and food intake were measured daily until a stable baseline was obtained. Mice received either vehicle (PBS) or 100 μg of LPS (Escherichia coli 0111:B4; Sigma, St. Louis, MO) in PBS via shallow midline, midabdominal, intraperitoneal injection. In our initial dose-response series, 100 μg of LPS reliably induced weight loss after administration without mortality. To determine the effect of COX inhibition, mice were pretreated with either vehicle (PBS plus 1% Tween 80) or vehicle plus agent 30 min before intraperitoneal injection of LPS or vehicle and were redosed at 8 and 24 h after LPS. COX inhibitors were administered via gavage by use of olive-tipped needles. Food intake and weight loss were assessed in mice treated with the nonselective COX inhibitor indomethacin at 150 μg/dose (~5 mg/kg), the COX-1-selective inhibitor SC-560 (Monsanto, St. Louis, MO) at 300 μg/dose (10 mg/kg), and the COX-2-selective inhibitor SC-236 (Monsanto) at 300 μg/dose (10 mg/kg). Doses for the selective inhibitors were determined on the basis of their reported IC50 values for inhibition of COX-1 or COX-2 relative to indomethacin (22, 28, 35). These doses have been demonstrated to be both isoform selective and efficacious in vivo in our previous studies (11, 32) and in those of others (22, 28, 35).

Plasma corticosterone, leptin, glucose, and IL-6 measurements. Plasma for measurement of corticosterone, leptin, glucose, and IL-6 was obtained from singly housed male C3H/HeN mice (n = 3–5 per group) by rapid retroorbital phlebotomy into heparinized capillary tubes, with a total time from first handling of the animal to completion of bleeding not exceeding 30 s. The baseline t = 0 sample was obtained at 1000 in ad libitum-fed mice. At 1400, mice were administered vehicle or SC-236 via gavage, and food was removed from the cage for the remainder of the experiment. Mice underwent intraperitoneal injection of vehicle or LPS 30 min after vehicle or SC-236 treatment. Additional blood samples were then collected 3 and 16 h after the LPS injection in the fasted animals. Blood was collected on ice, and plasma was separated by centrifugation and stored at ~80°C until assay. Plasma concentration of corticosterone (ICN Biomedicals, Costa Mesa, CA) was determined by radioimmunoassay as previously described (3, 13). Plasma concentrations of IL-6 (Pharmingen, San Diego, CA) and leptin (R & D Systems, Minneapolis, MN) were measured by ELISA per the manufacturer’s instructions. Plasma glucose concentration was measured on a HemoCue blood glucose analyzer (HemoCue, Mission Viejo, CA).

Hypothalamic in situ mRNA hybridization. Hypothalami were isolated from C3H/HeN male mice (n = 3 per group) pretreated with either vehicle or SC-236, as described above, at 1400, followed by injection of vehicle or LPS and food withdrawal for 16 h. After 24-h immersion in 4% paraformaldehyde, hypothalami were cryoprotected in 10% sucrose in diethyl pyrocarbonate (DEPC)–PBS. Samples were embedded in OCT (Sakura Finetek USA, Torrance, CA), cut into 10-μm sections on a cryostat, and thaw-mounted onto Superfrost plus slides (Fisher Scientific, Pittsburgh, PA). In situ hybridization utilized an [α-35S]UTP-labeled 320 base anti-sense riboprobe from the mouse corticotropin-releasing hormone (CRH) gene or a 511 base anti-sense riboprobe from the rat neuropeptide Y (NPY) cDNA (generously provided by Dr. Steven Sabol, National Heart, Lung, and Blood Institute, National Institutes of Health) by methods previously described (27). For CRH and NPY mRNA detection, the regions of the hypothalamic paraventricular and arcuate nuclei were determined by Nissl staining and light microscopy. Six sections spanning each nucleus were hybridized to CRH (paraventricular nucleus) or NPY (arcuate nucleus) probes, and the two sections of peak intensity were imaged for densitometric analysis. Hybridizing probes were quantitated by exposure of slides to Hyperfilm βMax (Amersham Life Science, Arlington Heights, IL) with densitometric analysis employing NIH Image Software.

Statistical methods. All results are expressed as means ± SE. Statistical analysis was by ANOVA, with P ≤ 0.05 considered significant.

RESULTS

To determine the consequences of selective COX inhibition on weight loss and hypophagia after induction of systemic inflammation with LPS, we treated adult mice with LPS and selective or nonselective COX inhibitors. Administration of the COX-2-selective inhibitor SC-236, the COX-1-selective inhibitor SC-560, or the nonselective inhibitor indomethacin without administration of LPS resulted in no change in body weight relative to vehicle-treated control mice (Fig. 1A). In accord with previous studies (18), treatment with indomethacin tended to reduce the weight loss associated with LPS administration (Fig. 1B, P = 0.09 vs. vehicle/LPS at 1 day after treatment). Selective attenuation of COX-2 activity with SC-236 significantly attenuated weight loss compared with mice treated either with vehicle and LPS or with the SC-560 and LPS (Fig. 1B). One day after LPS treatment, weight loss of mice receiving SC-236 was ~50% of the vehicle/LPS and SC-560/LPS groups. The difference in weight loss between LPS-treated SC-236 and SC-560 groups remained significant at 2 and 3 days after LPS treatment. Additionally, the SC-560/LPS group exhibited sustained weight reduction compared with the vehicle/LPS group at 3 days after treatment, suggesting that inhibition of COX-1 during systemic inflammation may be detrimental.

To further confirm the differential effects of COX-1 vs. COX-2-derived prostaglandins on weight loss after systemic immune activation, we evaluated WT, COX-1,
and COX-2 KO mice after LPS administration. For these experiments, female mice were used because COX-2 KO male mice have early mortality (26), precluding acquisition of adequate numbers of otherwise healthy adult COX-2 KO males for our studies. In accord with the above experiments utilizing COX-selective inhibitors, COX-2 KO mice demonstrated significantly less weight loss than COX-1 KO mice 1 day after LPS administration ($P < 0.01$; Fig. 2) and tended to have less weight loss than WT mice at this time point as well ($P = 0.08$). At 2 days after LPS administration, COX-2 KO mice continued to exhibit significantly better preservation of weight than COX-1 KO mice.

Changes in either energy intake or energy utilization could contribute to the attenuation in weight loss associated with selective COX-2 inhibition or deficiency. To evaluate the effect of COX-2 inhibition on appetite, we measured food intake in LPS-treated mice with or without simultaneous COX-2 inhibition by SC-236 (Fig. 3A). Administration of LPS alone resulted in a 60% reduction in food intake compared with vehicle-treated mice over the 24-h period after induction of

![Graph](image1.png)

**Fig. 1.** Pharmacological prostaglandin synthesis inhibition attenuates lipopolysaccharide (LPS)-induced weight loss. A: body weight changes after administration of vehicle ($n = 7$), indomethacin (Indo, $n = 4$), or isoform-selective cyclooxygenase (COX) inhibitors ($n = 4–5$) without LPS administration. B: body weight changes after administration of vehicle ($n = 11$), Indo ($n = 5$), or isoform-selective COX inhibitors ($n = 10–12$) with LPS administration. Statistically significant changes: day (d) 1, $P < 0.001$, vehicle/LPS vs. SC-236/LPS; $P < 0.05$, SC-236/LPS vs. SC-560/LPS; day 2, $P < 0.05$ SC-236/LPS vs. SC-560/LPS; day 3, $P = 0.01$, vehicle or SC-236/LPS vs. SC-560/LPS.

![Graph](image2.png)

**Fig. 2.** Genetic prostaglandin synthesis inhibition attenuates LPS-induced weight loss. COX-2 knockout (KO) mice tended to have less weight loss than wild-type (WT) mice 1 day after LPS administration ($P = 0.08$) and significantly less weight loss than COX-1 KO mice both 1 day ($P < 0.01$) and 2 days ($P < 0.01$) after LPS administration ($n = 5–7$ per group).

![Graph](image3.png)

**Fig. 3.** Appetite and metabolic rate changes after LPS administration with COX-2 inhibition. A: food intake over the 24-h period after LPS administration. *$P < 0.01$ vs. vehicle/LPS and vehicle/vehicle ($n = 9–10$ per group). B: body weight changes with overnight food withdrawal and LPS administration ($n = 5$ per group). *$P < 0.005$ vs. vehicle/vehicle; **$P < 0.05$ vs. vehicle/vehicle.
systemic immune system activation. In contrast, SC-236 administration to LPS-treated animals attenuated the hypophagic response, with food intake increasing by 50% compared with mice receiving LPS alone. Consistent with the attenuation of weight loss after LPS, SC-236 attenuated, but did not completely block, the hypophagic response. In further support of the observation that the effect of SC-236 in reducing weight loss depends on maintenance of food intake rather than changes in energy utilization, mice that were treated with vehicle or SC-236 and then fasted for 16 h after LPS administration demonstrated identical weight loss (Fig. 3B).

Maintenance of body weight, food intake, and overall nutritional status in the setting of acute bacterial infection or chronic inflammatory diseases would be of substantial health benefit. However, if maintenance of food intake and body weight after LPS occurred as a consequence of inhibition of the cytokine response to inflammation, the ability of the organism to combat infection and not succumb to sepsis might be compromised. To determine the consequences of COX-2 inhibition on cytokine production, we measured plasma IL-6 concentration after LPS administration. Normal IL-6 production in response to LPS requires the prior induction of both TNF-α and IL-1β, and IL-6 remains elevated for a prolonged period, providing a useful marker for integration of the cytokine response (1, 31, 40). As expected, IL-6 rose dramatically after LPS administration (Fig. 4A). Administration of SC-236 in conjunction with LPS resulted in no change in the magnitude of early induction of IL-6 as measured 3 h after LPS, or in the sustained increases in IL-6 at 16 h after LPS.

As additional evidence that the relative specificity of the consequences of COX-2 inhibition after LPS was on the hypophagic response rather than the overall cytokine and inflammatory response, we evaluated activity of the hypothalamic-pituitary-adrenal axis. Stimulation of adrenal glucocorticoid release during inflammation reflects actions of cytokines at central nervous system, pituitary, and adrenal sites and provides a useful integrated measure of stimulation and damping of the inflammatory response (3, 5, 23, 38). LPS administration resulted in marked elevation of plasma corticosterone concentrations at 3 h that was sustained at 16 h after administration (Fig. 4B). A small increase in plasma corticosterone was observed at the 3rd h in mice that did not receive LPS, because this time point coincided with the normal circadian peak in glucocorticoid production. There was no difference in the magnitude or duration of adrenal activation in those mice treated with LPS and SC-236. In accord with the sustained increase in adrenal glucocorticoid output after LPS, CRH mRNA in the paraventricular nucleus of the hypothalamus was elevated about threefold in both LPS and SC-236/LPS groups (Fig. 5).

To determine whether COX-2-generated prostaglandins had direct effects on appetite or acted indirectly on appetite centers via modulation of neuropeptides or other molecules, we measured NPY mRNA in the ar-
DISCUSSION

The data presented in this study demonstrate that selective pharmacological or genetic blockade of COX-2-generated prostaglandins effectively attenuates the hypophagic response to systemic inflammation induced by LPS. Unlike other agents that attenuate inflammation-induced anorexia, such as the phosphodiesterase inhibitor pentoxiphylline, calcium channel blockers, and glucocorticoids that block proximal events in the response to LPS (17, 18, 30), COX-2-selective drugs decrease anorexia without diminishing the cytokine response. Previous studies have demonstrated that NSAIDs may actually increase, rather than decrease, proximal events in the response during inflammation, such as induction of TNF-α (25), and our data extend this analysis by demonstrating no intermediate- to long-term reduction in IL-6 production. Because we find that COX-2 inhibition does not alter the expression of anorexigenic neuropeptides such as CRH, orexigenic neuropeptides such as NPY, or leptin after LPS administration, COX-2-generated prostaglandins are likely to directly modulate central appetite centers. Alternatively, COX-2 could still indirectly modulate appetite by action on one of several neuropeptide pathways not evaluated in the present study.

After LPS administration, it has previously been found that COX-2 mRNA and protein are induced within the central nervous system (CNS), predominantly in vascular endothelium, but also in specific neuronal populations (4, 24) as well as peripheral tissues (9, 11, 22, 26). Prominent induction of COX-2 mRNA has been noted specifically within the parenchyma of the hypothalamic paraventricular nucleus (15). In accord with the effects we observed on food intake after LPS administration, COX-2 has also been demonstrated to be the COX isoform essential for generation of the febrile response after systemic inflammation (20). Although impairing fever and concomitant increases in metabolic rate could be postulated as contributors to the attenuated weight loss after COX-2 inhibition, the equivalent weight loss we found in fasted mice treated with LPS and vehicle or SC-236 suggests that the primary action of COX-2 inhibition is modulation of food intake rather than metabolic rate. Surprisingly, LPS-treated mice demonstrated less weight loss than vehicle-treated controls. This finding may reflect decreased activity and caloric expenditure in the LPS-treated animals or decreased metabolic rate after LPS administration. Because plasma glucose concentration decreases after LPS administration, insulin resistance resulting in impaired glucose utilization is unlikely to be an important contributor to LPS-induced weight loss. Furthermore, selective COX-2 inhibition does not alter the severity of hypoglycemia after LPS, suggesting that COX-2 inhibition does not promote food intake by causing increased hypoglycemic drive.

The partial, rather than complete, attenuation of food intake and weight loss that we found with nonselective or COX-2-selective inhibition suggests that prostaglandin-independent pathways are also involved in the anorectic response to inflammation. The TNF-α response to LPS is not inhibited by administration of indomethacin and could still contribute to early decreases in food intake by direct CNS actions. Additionally, partially redundant actions of prostaglandins and cytokines other than TNF-α, which remain elevated for a longer period of time, may result in continued diminu-

Fig. 5. In situ hybridization analysis of hypothalamic corticotropin-releasing hormone (CRH) and neuropeptide Y (NPY). A: representative autoradiograms of coronal sections through the hypothalamic paraventricular nucleus (pvn) for CRH and arcuate nucleus (arc) for NPY mRNA detection. Low-magnification images of entire coronal sections (left panels for each probe) and high-magnification images of paraventricular or arcuate nuclei for CRH and NPY hybridizations, respectively (right boxed panels for each probe), are shown. The central nucleus of the amygdala (am) also demonstrates CRH mRNA hybridization. V, vehicle. B: quantitative densitometric analysis of CRH and NPY in situ autoradiograms (n = 3 per group). *P < 0.05 vs. vehicle/vehicle CRH.

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ished intake after prostaglandin inhibition. One cytokine that is likely to be capable of inhibiting food intake and independent of prostaglandins is IL-6. Although IL-6 KO mice have comparable decreases in food intake compared with WT mice in response to LPS, IL-6 KO mice have preserved food intake in response to local inflammation in response to turpentine abscess formation (8). These findings suggest that IL-6 has the capacity to suppress food intake, but in the setting of systemic inflammation induced by LPS administration, there are redundant factors capable of appetite suppression that are generated such that IL-6 is not essential. When considered together with our studies, this redundancy includes prostaglandins. Evaluation of the response of IL-6 KO mice to LPS and prostaglandin synthesis inhibition would be informative in this regard.

In contrast to the selective inhibition of COX-2, selective inhibition of COX-1 during inflammation appears detrimental. In our studies, inhibition or genetic inactivation of COX-1 prolongs the time until recovery of baseline body weight after LPS injection, and combined inhibition of COX-1 and COX-2 is not as effective as selective COX-2 inhibition in attenuating weight loss. In accord with these observations, selective inhibition of COX-1 had little or no effect on local prostaglandin production induced by LPS in an air pouch model system or foot pad edema induced by carrageenan injection, but it did significantly reduce prostaglandin production by the gastric mucosa (35). Because transient COX-1 inhibition in control mice that did not receive LPS is not associated with weight loss, our findings suggest that COX-1-generated prostaglandins may serve a protective role during inflammation, such as maintaining gastric mucosal integrity in the face of fasting and stress. This notion is strongly supported by recent studies demonstrating that selective COX-1 inhibition alone does not result in gastric damage, whereas combined COX-1 and COX-2 inhibition, or COX-1 inhibition associated with acid challenge or glucocorticoid administration, does (10, 39). The majority of NSAIDs in common clinical use for fever control during infection demonstrate substantial COX-1 inhibitory activity and are likely to cause similar decreases in food intake and body weight maintenance during the recovery phase of illness. The ability to diminish COX-2-derived prostaglandins selectively during inflammation or sepsis will maximize the beneficial actions of inhibiting inflammation-induced prostaglandins for fever control and food intake, maintain cytokine action, and minimize detrimental actions important for recovery from illness imparted by COX-1.

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