Cyclooxygenase-1 or -2—which one mediates lipopolysaccharide-induced hypothermia?

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1Systemic Inflammation Laboratory, Trauma Research, St. Joseph’s Hospital and Medical Center, Phoenix, Arizona; 2Department of Pharmaceutical Sciences, Albany College of Pharmacy and Health Sciences, Albany, New York; 3Department of Chemistry and Biochemistry, Brigham Young University, Provo, Utah; and 4Neurology and Neurosurgery Research, Barrow Neurological Institute, St. Joseph’s Hospital and Medical Center, Phoenix, Arizona

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Steiner AA, Hunter JC, Phipps SM, Nucci TB, Oliveira DL, Roberts JL, Scheck AC, Simmons DL, Romanovsky AA. Cyclooxygenase-1 or -2—which one mediates lipopolysaccharide-induced hypothermia? Am J Physiol Regul Integr Comp Physiol 297: R485–R494, 2009. First published June 10, 2009; doi:10.1152/ajpregu.91026.2008.—Systemic inflammation is severe enough to compromise tissue perfusion or threaten energy reserves (7, 82). Whereas the biological value of fever is thought to be related to its immunostimulant and antibacterial effects (43), the biological value of hypothermia may be related to energy conservation when inflammation is severe enough to compromise tissue perfusion or threaten energy reserves (7, 82).

There is no doubt that cyclooxygenase (COX) plays a critical role in the genesis of fever by catalyzing the conversion of arachidonic acid to prostaglandin (PG) H2, the immediate precursor of febrile prostaglandin PG2 (10, 35, 55, 66). Clinical fevers (5) and all phases of experimental, LPS-induced fever (13, 49, 81, 83, 90) are thought to be mediated by COX-2, the inducible isofrm, and not by COX-1, the predominantly constitutive isoform. An involvement of COX in LPS-induced hypothermia has also been suggested (7, 19, 86), and it is believed to be associated with the formation of potentially cryogenic PG2 from COX-derived PGH2 (86). However, studies of the COX isoforms involved in LPS hypothermia have yielded contradictory results. Dogan et al. (20) and Akarsu and Mamuk (2) reported a suppression of LPS hypothermia by a preferential (valeryl-salicylate) or selective (SC-560) COX-1 inhibitor in rats, thus suggesting that this response is mediated by COX-1. Zhang et al. (90) reported the opposite, hypothermia-enhancing effect of SC-560 in the rat, thus suggesting that products of COX-1 inhibit LPS hypothermia in the same species. Further complicating the picture, Dogan et al. (20, 21) and Zhang et al. (90) found that a preferential (nimesulide) or selective (SC-236) inhibitor of COX-2 attenuated LPS hypothermia in rats, thus suggesting mediation by COX-2.

The present study was conducted to clarify which COX isofrm, if any, mediates hypothermia in systemic inflammation. We compared the effects of SC-560 and SC-236 on the Tb responses of rats to different doses of LPS at different Ta’s. Because Tb responses to high doses of LPS are associated with hypotension (46, 62, 70), arterial blood pressure was monitored in a subset of experiments. As a follow up to our finding that
the COX-1 inhibitor, but not the COX-2 inhibitor, blocked LPS-induced hypothermia, we measured the expression of COX-1 at the mRNA and protein levels and the functional activity of the COX-1 pathway at the onset of the LPS-induced responses.

METHODS

Animals

The study was conducted in male Wistar rats (Harlan, Indianapolis, IN) that weighed 290–380 g at the time of experiments. Initially, the rats were housed three per standard cage; after surgery, they were housed individually. The cages were kept in a rack equipped with a Smart Bio-Pack ventilation system (model SB4100) and Thermo-Pak temperature control system (model TP2000; Allentown Caging Equipment, Allentown, NJ); the temperature of the incoming air was maintained at 28°C. Standard rat chow and tap water were available ad libitum. The room was on a 12:12-h light-dark cycle (lights on at 7:00 AM). The cage space was enriched with artificial “rat holes” (cylindrical conifers made of stainless-steel wire). In addition to spending time in the conifers voluntarily, the rats were systematically habituated to being located in the conifers (7 daily training sessions, 4 h each). The same conifers were used later in the experiments. Rodents are readily adaptable to confinement to an extent that habituated rodents respond to it with neither stress fever (71) nor other signs of stress (1, 32, 56, 78). Each rat was used in an experiment once and euthanized with sodium pentobarbital (100 mg/kg iv) immediately thereafter. All procedures were conducted under protocols approved by the St. Joseph’s Hospital and Medical Center’s Animal Care and Use Committee.

Surgical Preparation

Four days before an experiment, every rat was implanted with an intravenous catheter; some rats were also implanted with intra-arterial catheters. The procedures were performed under ketamine-xylazine anesthesia and antibiotic (enrofloxacin, 1.1 mg/kg sc) protection. During surgery, a rat was maintained on a board warmed to 37°C by a Deltaplace isothermal pad (Braintree Scientific, Braintree, MA).

For venous catheterization, a small longitudinal incision was made on the left ventral surface of the neck. The left jugular vein was exposed, freed from its surrounding connective tissue, and ligated. A silicone catheter (ID 0.5 mm, OD 0.9 mm) filled with heparinized (10 U/ml) saline was passed into the superior vena cava through the jugular vein and secured in place with ligatures. The free end of the catheter was knotted, tunneled under the skin to the nape, and exteriorized. The skin was sutured.

For arterial catheterization, the right ventral surface of the neck was incised, and the right carotid artery was isolated and clamped by a microclip. The tip of a PE-50 catheter (ID 0.6 mm, OD 1.0 mm) filled with heparinized saline was placed into the artery, the clip was removed, and the catheter was moved toward the aorta. The catheter was secured in place with ligatures. The free end of the catheter was heat-sealed and exteriorized at the nape. The skin was sutured.

To prevent postsurgical hypothermia, the animals were allowed to recover from anesthesia in an environmental chamber (model 3940; Forma Scientific, Marietta, OH) set to 28.0°C. The intravenous catheters were flushed with heparinized saline every other day; the intra-arterial catheters were flushed daily.

Experimental Setup

On the day of the experiment, each rat was placed in a confiner. For measurement of colonic temperature (an index of $T_b$), a copper-constantan thermocouple was inserted in the colon, 10 cm beyond the anal sphincter. The thermocouple was fixed to the base of the tail with adhesive tape and plugged into a data logger (Cole-Parmer, Vernon Hills, IL), which conveyed the data to a personal computer. The rat was transferred to an environmental chamber (Forma Scientific) set to either a neutral (30.0°C) or subneutral (22.0°C) $T_a$ (67). The venous catheter was extended with a length of PE-50 tubing filled with saline, and the extension was passed through a wall port and connected to a syringe filled with the drug of interest. This setup permits intravenous drug administration without disturbing a rat and without causing a marked stress response that often presents a major limitation in thermoregulation experiments (69, 73).

If present, the arterial catheter was used for recording arterial pressure. A PE-50 tubing extension of the arterial catheter was passed through a wall port and connected to a differential pressure transducer (Columbus Instruments, Columbus, OH). The analog output of the transducer was converted by the Datamax logger interface (Columbus Instruments) into a digital signal, which was fed into a personal computer. The pulsatile arterial pressure data were collected and processed using the Datamax software (Columbus Instruments). Mean arterial pressure was calculated from a time integral of the pulsatile pressure.

Drug Administration

COX inhibitors. Selective COX-1 and COX-2 inhibitors (SC-560 and SC-236, respectively) were gifts from Pfizer (Groton, CT). In vitro studies demonstrate that SC-560 is ~700 times more potent to inhibit COX-1 than COX-2 (76), whereas SC-236 is ~1,800 times more potent to inhibit COX-2 than COX-1 (65). SC-560 and SC-236 were dissolved in ethanol to a final concentration of 16 and 8 mg/ml, respectively. These solutions were aliquoted and stored at −80°C until the day of the experiment. On the day of the experiment, an aliquot was warmed to room temperature, and infused intravenously at a rate of 31 μg/kg/min for 10 min. Control rats were infused with the vehicle at the same low rate. This infusion protocol produced neither hemolysis (determined based the color of the plasma) nor other signs of ethanol toxicity. The doses of SC-560 and SC-236 delivered over the 10-min infusion were 5 and 2.5 mg/kg, respectively. At these in-vivo doses, SC-560 maximally inhibits COX-1 without affecting COX-2, whereas SC-236 maximally inhibits COX-2 without affecting COX-1 (31, 53, 54).

LPS. E. coli 0111:B4 LPS was purchased from Sigma-Aldrich (St. Louis, MO). A stock suspension of LPS (5 mg/ml) in pyrogen-free saline was stored at −20°C. On the day of the experiment, the stock was diluted to a final concentration of either 10 or 1,000 μg/ml. The diluted LPS suspension or saline was bolus injected (1 ml/kg) through the extension of the venous catheter 20 min after completion of the 10-min-long infusion of SC-560, SC-236, or their vehicle. The resultant doses of LPS (10 or 1,000 μg/kg iv) have been repeatedly shown to cause a mild polyphasic fever (the lower dose) or a brief hypothermia followed by fever (the higher dose) at a neutral $T_a$, whereas they cause a dose-dependent hypothermia at a subneutral $T_a$ (68–71, 79).

Functional Activity of the COX-1 Pathway and COX-1 Expression

COX-1 pathway activity was assessed on the basis of the ex vivo production of PGE2 that is blocked by SC-560. We selected the COX-1-mediated synthesis of PGE2 as a measure of the functional activity of the COX-1 pathway because the immediate product of the reaction catalyzed by COX-1, PGH2, is unstable. Among the multiple products synthesized in the next step (by several PGE, D, F, and I synthases and by thromboxane synthases), PGE2 is reasonably stable and the most robustly produced during inflammation in a wide spectrum of organs and tissues throughout the body (35). Furthermore, at least in some situations, the critical, rate-limiting step of inflammation-associated PGE2 synthesis seems to be the one catalyzed by COX and not the one catalyzed by terminal synthases (8). If one accepts that LPS-induced hypothermia is mediated by PGE2 (which may not be the case; see Refs. 27, 44), an alternative approach
would be to use the COX-1-mediated PGD2 synthesis as a measure of COX-1 pathway activity. However, PGD2 is much less stable than PGE2, whereas some more stable products of PGD2, such as 15-deoxy-Delta12,14-PGJ2, increase (rather than decrease) deep Tb in rats (A. A. Steiner, A. S. Dragic, J. Pan, A. A. Romanovsky; unpublished observation). Hence, the stability of PGE2 and the robustness of its synthesis under inflammatory conditions provide a solid justification for the use of COX-1-mediated PGE2 synthesis as a measure of COX-1 pathway activity. It should be understood, however, that this measure reflects the enzymatic activity not only of COX-1, but also that of several PGE terminal synthases, and depends both on how COX-1 is coupled with each synthase and on which enzyme in each COX-1-synthesase pair catalyzes the critical step.

Tissues for the functional activity assay were harvested from rats 50 min after injection of LPS (1,000 μg/kg) or saline at a Ta of 22.0°C. This time point corresponds to the maximum rate of fall in Tb during LPS hypothermia. At the time of tissue harvesting, rats were anesthetized intravenously with ketamine-xylazine-acepromazine (5.6, 0.6, and 0.1 mg/kg, respectively). Following transcardiac perfusion with 30 ml of saline (10 ml/min), the entire brain, right kidney, spleen, right lung, and the central lobe of the liver were collected. Each tissue was rinsed with PBS (0.01 M, pH 7.4) and transferred to a polypropylene conical tube. PBS was added to each tube to achieve a PBS:tissue ratio of 5:1 (wt:wt), and the tissue was then homogenized on ice using an ultrasonic cell disruptor. Aliquots of the homogenate were preincubated (25°C, 15 min) with or without SC-560 (0.6 μM). At this concentration, SC-560 inhibits COX-1 activity by >95% (76). Preincubation was followed by incubation (37°C, 10 min) of the homogenate with arachidonic acid (30 μM). Enzymatic reactions were stopped by heating the homogenate to 65°C for 5 min. The homogenate was then centrifuged (13,000 g, 10 min, 4°C), and the supernatant and pellet were stored separately at −80°C. The supernatant was assayed for PGE2 by radiommunoassay using a rabbit antibody raised against a PGE2-albumin complex (Sigma-Aldrich) and for total protein by the Bradford method (Bio-Rad, Hercules, CA); the assays were conducted according to the manufacturers’ instructions with 30 ml of saline (10 ml/min), the entire brain, right kidney, spleen, right lung, and the central lobe of the liver were collected. Each tissue was rinsed with PBS (0.01 M, pH 7.4) and transferred to a polypropylene conical tube. PBS was added to each tube to achieve a PBS:tissue ratio of 5:1 (wt:wt), and the tissue was then homogenized on ice using an ultrasonic cell disruptor. Aliquots of the homogenate were preincubated (25°C, 15 min) with or without SC-560 (0.6 μM). At this concentration, SC-560 inhibits COX-1 activity by >95% (76). Preincubation was followed by incubation (37°C, 10 min) of the homogenate with arachidonic acid (30 μM). Enzymatic reactions were stopped by heating the homogenate to 65°C for 5 min. The homogenate was then centrifuged (13,000 g, 10 min, 4°C), and the supernatant and pellet were stored separately at −80°C. The supernatant was assayed for PGE2 by radiommunoassay using a rabbit antibody raised against a PGE2-albumin complex (Sigma-Aldrich) and for total protein by the Bradford method (Bio-Rad, Hercules, CA); the assays were conducted according to the manufacturers’ instructions. The concentrations of PGE2 and total protein were used to calculate the COX-1 pathway activity (PGE2 concentration/protein concentration/duration of arachidonic acid incubation). The COX-1 pathway activity was calculated by subtracting the ex vivo activity of a sample incubated in the absence of SC-560 from the activity of each sample incubated in the presence of SC-560.

In view of the fact that LPS increased the functional activity of the COX-1 pathway in spleen but not in other tissues (see RESULTS), the expression of COX-1 was measured in spleen samples only. The pellets from the samples processed for functional activity were subjected to Western blot analysis for determination of COX-1 protein content, as Western blot analysis for determination of COX-1 protein content, as described in detail elsewhere (34). RNA integrity was determined by a 2100 Bioanalyzer (Agilent, Santa Clara, CA). Total RNA was reverse transcribed to cDNA by random hexamer priming using SuperScript III First-Strand Synthesis System (Invitrogen). For quantitative real-time PCR, a LightCycler (Roche Applied Science, Indianapolis, IN) was used. The concentration of double-stranded DNA amplicon was monitored using Light Cycler FastStart DNA Master Plus SYBR Green I (Roche Applied Science). Primers for COX-1 (gene of interest) were 5'-ACTGGAAACCCACGACACATTC (forward) and 5'-ACTCTCCCTCCAGAAGACG (reverse); annealing temperature was 62°C. Primers for β-actin (housekeeping gene) were 5'-CGAGTCGCGTCCCCAGCGGA (forward) and 5'-GAGCGAGCCGGCCGATATC (reverse); annealing temperature was 62°C. The relative expression R of the gene of interest was calculated according to the formula: \( R_i = 2^{(\Delta\Delta C_t)} \), where \( N \) is the threshold cycle number, i.e., the number of the amplification cycle in which fluorescence of a given sample becomes significantly different from the baseline signal (36). The indexes \( i \) and \( h \) refer to the gene of interest and housekeeping gene, respectively; the index \( t \) refers to individual samples from rats treated with either LPS or saline; and the index \( c \) refers to control samples (namely, samples pooled from untreated rats). This equation is based on the inverse proportionality between \( N \) and \( \log_2 C \), where \( C \) is the initial template concentration in the PCR sample. The physical meaning of \( R_i \) is the concentration of mRNA of interest (COX-1) in a sample from a treated (with LPS or saline) animal divided by the concentration of the same message in the simultaneously run untreated controls, in which each concentration is normalized for the concentration of a housekeeping mRNA (β-actin) in the same sample. Gene amplification was verified by running agarose gel electrophoresis of each amplicon obtained during the exponential phase of PCR amplification.

**Statistical Analyses**

The Tb and blood pressure responses were compared across treatments and time points by a two-way ANOVA. The data on COX-1 pathway activity were compared across treatments and organs by a two-way ANOVA. The COX-1 protein and mRNA levels in the spleen were compared across treatments by Student’s t-test. All analyses were performed using Statistica Advanced 8.0 (StatSoft, Tulsa, OK). The data are reported as means ± SE.

**RESULTS**

LPS-induced hypothermia is blocked by SC-560 but enhanced by SC-236. We studied the effects of a COX-1 inhibitor (SC-560), a COX-2 inhibitor (SC-236), or their vehicle on the thermoregulatory responses of rats injected with a lower dose of LPS (10 μg/kg), a higher dose of LPS (1,000 μg/kg), or saline at a neutral (30°C) or subneutral (22°C) Tb. Baseline Tb ranged from 37.5 to 38.5°C; values near the upper end of the range were recorded at the neutral Tb, whereas values near the lower end of the range were recorded at the subneutral Tb. Regardless of Tb, no thermoregulatory response was observed in the saline-treated rats pretreated with SC-560, SC-236, or their vehicle (Figs. 1 and 2). At the neutral Tb (Fig. 1), the vehicle-pretreated rats responded to the lower dose of LPS with a typical polyphasic fever, which consisted of three consecutive Tb peaks rising at −50, 120, and 300 min. All phases of
Because the hypothermic response to LPS has been reported to be associated with hypotension (46, 70), we studied the effects of SC-560 and SC-236 on the blood pressure changes caused by the higher dose of LPS. This experiment was performed at the subneutral Ta (22°C), because the effects of SC-560 and SC-236 on LPS hypothermia were most manifest at this Ta (Fig. 2). Baseline mean arterial pressure was \(~\sim 130\) mmHg, a value similar to those recorded by others in rats exposed to a subneutral Ta (15, 64). No significant change in mean arterial pressure was observed in the saline-treated rats pretreated with SC-560, SC-236, or their vehicle (Fig. 3). Injection of LPS (1,000 \(\mu\)g/kg) to the vehicle-pretreated rats evoked a decrease \((\sim 30\) mmHg) in blood pressure. The blood pressure reached a nadir at \(~\sim 80\) min after LPS injection, corresponding in time to the first phase of the hypothermic response. LPS-induced hypotension was largely attenuated by pretreatment with either SC-560 \((P < 2.2 \times 10^{-5}, 50–170\) min) or SC-236 \((P < 1.2 \times 10^{-4}, 50–120\) min), despite the
fact that these drugs had opposite effects on LPS-induced hypothermia.

**The Functional Activity of the COX-1 Pathway (but not COX-1 Expression) Is Increased by LPS**

The functional activity of the COX-1 pathway (COX-1-mediated PGE$_2$ synthesis ex vivo) was measured in tissues collected 50 min after intravenous administration of LPS (1,000 μg/kg) or saline at a subneutral $T_a$ (22°C). This time corresponds to the onset of LPS-induced hypothermia (and also hypotension). In the controls (saline-treated rats), the COX-1 pathway was active in all organs investigated: liver, lung, spleen, kidney, and brain (Fig. 4). Compared with saline, LPS did not significantly change the activity of the COX-1 pathway in the liver, lung, kidney, or brain. However, it produced a 3.6-fold increase in the COX-1 pathway activity in the spleen ($P < 7.5 \times 10^{-3}$) but not in the liver, lung, kidney, or brain.

We next evaluated whether the increased activity of the COX-1 pathway in the spleen was associated with changes in the splenic expression of COX-1 measured at the mRNA and protein levels. For both COX-1 and β-actin, a single mRNA product of the expected size (196 bp for COX-1 and 106 bp for β-actin) was amplified (Fig. 5A). No product was amplified in the absence of primers or when water was used instead of RNA (data not shown). The spleen mRNA expression of COX-1 (relative to β-actin) did not differ ($P = 0.16$) between LPS- and saline-treated rats (Fig. 5A). The Western blot analysis of COX-1 revealed a protein with a molecular mass between 70 and 80 kDa in the Western blot membranes of all spleen samples (Fig. 5B). The same molecular mass protein was markedly expressed in platelets (positive control for COX-1), and no such protein was revealed when the Western blot membranes were incubated without the anti-COX-1 antibody (negative control; data not shown). The intensity of the COX-1 bands did not differ ($P = 0.69$) between samples obtained from the spleens of LPS- and saline-treated rats (Fig. 5B). Taken together, the data indicate that the expression of COX-1 in the spleen did not change at the onset of LPS hypothermia (and hypotension).
DISCUSSION

Effects of COX-1 and COX-2 Inhibitors on LPS-Induced Responses

The present study was carried out to clarify which isoform of COX mediates hypothermia in systemic inflammation. First, we evaluated the effects of SC-560 (COX-1 inhibitor) and SC-236 (COX-2 inhibitor) on the thermoregulatory responses to LPS. SC-236 blocked all phases of LPS-induced fever in experiments conducted at a neutral $T_a$, whereas it enhanced LPS-induced hypothermia at a subneutral $T_a$. These findings indicate that COX-2 not only mediates fever but also limits the hypothermic response to LPS. It is possible that COX-2-mediated production of the same febrigenic mediator, possibly PGE$_2$ (57), underlies the involvement of this enzyme in both the development of fever and the limitation of hypothermia. Indeed, the complexity of the thermoregulatory response to LPS is thought to result from a balance between a febrile component (driven by febrigenic mediators) and a hypothermic component (driven by cryogenic mediators) (42, 82, 84).

The COX-1 inhibitor, SC-560, consistently blocked LPS hypothermia (but not fever) under all experimental conditions tested, thus indicating that COX-1 is required for the development of LPS hypothermia. The COX-1 product involved in LPS hypothermia has yet to be identified, but PGD$_2$ is a potential candidate, as it induces hypothermia when injected in rats either intracerebroventricularly (86) or intravenously: as an albumin complex (A. A. Steiner, A. S. Dragic, J. Pan, A. A. Romanovsky, unpublished observation) or as a hydroalcoholic solution (A. Garami, E. Pakai, A. A. Romanovsky; unpublished observation). It should be noted, however, that Krueger et al. (44) have reported that high doses of PGD$_2$ cause fever (rather than hypothermia) in rabbits; the same authors have suggested that effects of PGD$_2$ on thermoregulation (and sleep) may be species specific. While this study was in preparation, Gao et al. (27) reported that intracisternal PGD$_2$ did not cause hypothermia in rats and, in fact, caused a delayed fever, possibly by interfering with the transport of the febrigenic PGE$_2$ in the brain. Clearly, the search for the COX-1-derived mediator of hypothermia should continue.

In contradiction with the present findings are studies by Dogan et al. (20) and Zhang et al. (90), which reported that SC-236 blocked LPS hypothermia. In the study by Dogan et al. (20), such a blockade occurred only when the dose of SC-236 was substantially higher (40 mg/kg) than the doses (1–15 mg/kg) known to selectively block COX-2 in vivo (31, 53). At such a high dose, SC-236 might have inhibited COX-1, or it might have exerted COX-unrelated effects (39). Although the study by Zhang et al. (90) employed a lower dose of SC-236 (5 mg/kg), it was limited by marked stress hyperthermia following intraperitoneal drug administration, which overlapped substantially with the early hypothermic response to LPS, thus making the results difficult to interpret. Zhang et al. (90) reported another observation that seemingly contradicts the present results, i.e., that SC-560 might have prolonged LPS hypothermia. It should be considered, however, that Zhang et al. (90) used a low dose of LPS, which resulted in only minimal decreases in $T_b$ ($\sim$0.5°C) in both SC-560-treated and SC-560-untrated rats. In the present study, the usage of higher doses of LPS in a tightly controlled thermal environ-
upstream or downstream from COX-1 may affect the activity of the COX-1 pathway, since these mechanisms determine substrate availability to COX-1 and the fate of the COX-1 product, respectively (see Perspectives and Significance).

A splicing variant of COX-1 has been identified in canine tissues (16). Originally named COX-3, this variant is now most commonly referred to as COX-1b. Might COX-1b have contributed to the increased COX-1 pathway activity found in the present study? At present, the answer to this question appears to be negative. Although a COX-1b transcript (which retains intron-1) has been found in rat tissues (40, 41), a functional COX-1b protein has not yet been identified in this species (77). The rat intron-1 (unlike the canine intron-1) contains an incomplete codon sequence consisting of 32 codons plus 2 extra nucleotides; the extra nucleotides are available to form a codon with the nucleotide of the exon that follows, thus shifting the reading frame of the exonic portion of the transcript (77).

Study Limitations and Design Considerations

In the present study, baseline colonic temperature in confined rats ranged from 37.5 to 38.5°C. These values are higher (by ~0.5°C) than baseline values of abdominal temperature recorded telemetrically in freely moving rats (see, for example, Refs. 2 and 90). However, this expected difference likely stems from two factors (for detailed analysis, see Ref. 71). First, colonic temperature is one of the highest temperatures in the rat’s body. It is 0.1–0.8°C higher than aortic temperature (22), and it is very likely higher than abdominal temperature, especially if the latter is measured near the abdominal wall (71). Second, the perception that “normal” values of abdominal temperature range from 37.0 to 37.5°C originated from experiments performed at room temperature (see, e.g., Refs. 2, 90), which are usually subneutral for rats (67). At a subneutral Ta, basal Tb of rats in our experiment rarely exceeded 38.0°C. Only when rats were exposed to a neutral Ta (30°C) did their basal Ta reach 38.5°C, an observation that agrees with the known influence of Ta on basal Tb (28, 67, 71, 73, 89). Nevertheless, the presence of a low-grade confinement stress in our experiments cannot be ruled out completely, especially because the rats had a somewhat elevated basal arterial pressure (~130 mmHg). One can argue, however, that the increased blood pressure, which was observed at a subneutral Ta only, was due to mild cold exposure (15, 64), rather than confinement, to which the animals were well adapted.

In spite of the fact that we carefully chose the doses of SC-560 and SC-236 so that each drug was administered at the minimal dose that has been demonstrated to maximally inhibit one COX isoform without affecting the other in vivo (31, 53, 54), the effectiveness of our approach is subject to limitations inherent to any pharmacological approach, including the possibility that a drug may produce COX-unrelated (off-target) effects (12). The limitations of pharmacological approaches can be offset by the use of complementary approaches such as genetically modified mice. Indeed, mice genetically deficient of COX-1 or COX-2 have been instrumental in studies that established the role of COX-2 in the mediation of fever (49, 83). However, the early hypothermia, which was observed in the present study and that has been reported to occur in response to LPS in rats (70, 79), rabbits (88), and chicken (18), does not occur in mice (73). The early (starts within minutes) hypothermic response of rats occurring at LPS doses of 10 µg/kg and higher clearly differs from the late (starts at 4 h or later) and prolonged (lasts 12 h or more) hypothermic response that develops in mice injected with extremely high (10,000 µg/kg and higher) doses of LPS (38, 73, 75). Hence, mice cannot be used to study the early LPS hypothermia that is common for other species. Another limitation of our study is that it is unknown to what extent the data obtained can be generalized beyond the experimental conditions used: an LPS model of systemic inflammation in the rat.

Conclusion

In conclusion, by conducting a differential analysis of the effects of two highly selective COX inhibitors in several models of LPS fever and hypothermia, the present study indicates that COX-1, and not COX-2, is the isoform that mediates the hypothermic response to LPS, at least in the rat. By investigating the functional activity of the COX-1 pathway (COX-1-mediated PGE2 synthesis ex vivo) and COX-1 expression at the mRNA and protein levels, this study has also found that COX-1 pathway activity increases in the spleen at the onset of LPS hypothermia via mechanisms that do not involve transcriptional upregulation of COX-1. By evaluating the effects of COX inhibitors on blood pressure, this study has further shown that both COX isoforms are required for the hypotensive response to LPS to develop.

Perspectives and Significance

In the absence of transcriptional upregulation of COX-1 in the spleen, an increased splenic COX-1 pathway activity during LPS hypothermia may involve not only posttranslational activation of COX-1 (discussed above), but also the activation of mechanisms upstream or downstream from COX-1. The COX substrate, arachidonic acid, derives from the breakdown of membrane phospholipids by enzymes of the phospholipase (PL) A2 superfamily (35, 58). It is generally accepted that certain PLA2 enzymes may couple more favorably with one COX isoform than with the other. For example, cytosolic PLA2-α colocalizes with COX-1, but not with COX-2, in the Golgi apparatus of activated epithelial cells in vitro (29). Therefore, the activation [possibly by phosphorylation (50)] of cytosolic PLA2-α may direct arachidonic acid to COX-1 in preference to COX-2. The phosphorylated form of cytosolic PLA2 has been detected in the lungs as early as 40 min after intravenous administration of LPS (80).

The mechanisms downstream from COX determine which bioactive prostanoids are formed (35, 85). Recent studies (23, 34, 74) have shown that microsomal PGE synthase-1 (mPGES-1) is the enzyme responsible for the conversion of PGH2 into febrigenic PGE2 (mPGES-1). This enzyme is functionally coupled with COX-2 in marked preference to COX-1, presumably because both COX-2 and mPGES-1 are primarily compartmentalized in the perinuclear envelope (60). Massive (more than 1,000 fold) transcriptional upregulation of mPGES-1 occurs in the course of LPS-induced fever (23, 34). The terminal synthases involved in the hypothermia of systemic inflammation remain to be identified, but given the ability of PGD2 to cause hypothermia, at least according to some reports (86), it is reasonable to suspect that a PGD synthase (PGDS) may be involved. There are two known PGDS isoforms: lipocalin.
PGDS (L-PGDS) and hematopoietic PGDS (H-PGDS) (58). Whereas the LPS-induced production of PGD₂ in neural tissue appears to be dependent on COX-2 and L-PGDS (30), the early production of PGD₂ by activated macrophages and mast cells seems to depend on COX-1 and H-PGDS (59, 61). Interestingly, large amounts of H-PGDS are present in the rat spleen (37), the organ in which the COX-1 pathway was activated at the onset of LPS hypothermia. Furthermore, Feleder et al. (24, 25) have recently shown that splenectomy or splenic vein ligation enhances LPS fever, and they have proposed that LPS causes the synthesis of a cryogenic lipid in the spleen. Our ligations elucidate a COX-1 pathway in the spleen and the consequent production of PGD₂ or some other COX-1-mediated mechanism underlies LPS-induced hypothermia is a subject of future studies.

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