Am J Physiol Regul Integr Comp Physiol 289: R1253–R1257, 2005.
First published August 4, 2005; doi:10.1152/ajpregu.00371.2005.

Expanding the febrigenic role of cyclooxygenase-2 to the previously overlooked responses

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Submitted 26 May 2005; accepted in final form 28 July 2005

Steiner, Alexandre A., Alla Y. Rudaya, Jared R. Robbins, Alexander S. Dragic, Robert Langenbach, and Andrej A. Romanovsky. Expanding the febrigenic role of cyclooxygenase-2 to the previously overlooked responses. *Am J Physiol Regul Integr Comp Physiol* 289: R1253–R1257, 2005. First published August 4, 2005; doi:10.1152/ajpregu.00371.2005.—Previous studies on the role of cyclooxygenase (COX)-1 and -2 in fever induced by intravenous LPS have failed to investigate the role of these isoenzymes in the earliest responses: monophasic fever (response to a low, near-threshold dose of LPS) and the first phase of polyphasic fever (response to higher doses). We studied these responses in 96 mice that were COX-1 or COX-2 deficient (−/−) or sufficient (+/+). Each mouse was implanted with a temperature telemetry probe into the peritoneal cavity and a jugular catheter. The study was conducted at a tightly controlled, neutral ambient temperature (31°C). To avoid stress hyperthermia (which masks the onset of fever), all injections were performed through a catheter extension. The +/+ mice responded to intravenous saline with no change in deep body temperature. To a low dose of LPS (1 μg/kg iv), they responded with a monophasic fever. To a higher dose (56 μg/kg), they responded with a polyphasic fever. Neither monophasic fever nor the first phase of polyphasic fever was attenuated in the COX-1 −/− mice, but both responses were absent in the COX-2 −/− mice. The second and third phases of polyphasic fever were also missing in the COX-2 −/− mice. The present study identifies a new, critical role for COX-2 in the mediation of the earliest responses to intravenous LPS: monophasic fever and the first phase of polyphasic fever. It also suggests that no product of the COX-1 gene, including the splice variant COX-1b (COX-3), is essential for these responses.

prostaglandin E_2; cyclooxygenase-1b; cyclooxygenase-$1V_1$; cyclooxygenase-3; prostaglandin H_2 synthase; body temperature; thermoregulation; febrile phases

FEBRILE RESPONSE TO BACTERIAL LPS depends, among other factors, on the LPS dose. At thermoneutrality, several species, including the rat (32) and mouse (36), respond to a low, near-threshold dose of intravenous LPS with a monophasic fever–a single rise in deep body temperature (T_b) characterized by a relatively long latency, small magnitude, and short duration. As the dose increases, a single bolus injection of LPS causes a polyphasic fever. This response has a shorter latency, higher magnitude, and longer duration. Even more importantly, polyphasic fever consists of several sequential T_b rises, called febrile phases, at least three of which have been identified in the rat (33) and mouse (36). Different febrile phases are associated with different symptoms of the sickness syndrome (32) and may involve different biochemical and physiological mechanisms (9, 14, 24, 34, 45).

There is no doubt that cyclooxygenase (COX) plays a critical role in the production of fever by catalyzing the conversion of arachidonic acid to PGH_2, the immediate precursor of febrigenic PGE_2 (for review, see Ref. 16). This enzyme has two isoforms, COX-1 (predominantly constitutive) and COX-2 (predominantly inducible) (for review, see Ref. 25). Studies involving highly selective COX-1 and COX-2 inhibitors (3, 4, 8, 31, 48) or genetically modified mice (22) have shown that COX-2, but not COX-1, mediates LPS fever at its later stages, which likely correspond to the second and third phases of the typical polyphasic febrile response. However, all studies referenced above involved a stressful, often painful, procedure of LPS administration; such a procedure led to the development of stress hyperthermia and thus masked the first phase of polyphasic fever. Most of those studies were also conducted at a poorly controlled ambient temperature, a condition that made differentiation of febrile phases impossible or unreliable, and none of those studies used LPS doses small enough to produce a monophasic fever. As a result, the two earliest responses to LPS, viz., monophasic fever and the first phase of polyphasic fever, were overlooked. The only study (42) aimed at investigating the involvement of COX-1 and COX-2 in these early responses to LPS was conducted in guinea pigs. However, results obtained in that study are difficult to interpret, because nimesulide, the drug used as a selective COX-2 inhibitor, is less selective than the COX-2 inhibitors of the new generation (30). Furthermore, nimesulide can affect febrile pathogenesis by acting on many targets other than COX (2). Hence, it remains to be determined which COX isoform mediates monophasic fever and the first phase of the polyphasic febrile response to LPS.

It is possible that the early responses to LPS are mediated not by COX-2, but by COX-1. An involvement of hepatic COX-1 in the initiation of the febrile response to systemic LPS has recently been proposed (28), and it has been demonstrated

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that COX-1 can play a role in various inflammatory responses, especially during their initial stages, when COX-2 is presumably not fully upregulated (12, 21, 43). To determine which COX isoform is involved in monophasic fever and the first phase of polyphasic fever were the aims of the present study. To meet these aims, we used mice genetically deficient in either COX-1 or COX-2 and applied the recently developed methodology of studying monophasic and polyphasic fever responses to intravenous LPS in this species (36).

MATERIALS AND METHODS

Animals. The present study was performed on 96 male mice with (−/−) or without (+/+) a homozygous, targeted, null mutation in either the COX-1 (21) or COX-2 (23) gene. All mice were obtained from Taconic Farms (Germantown, NY). The COX-1 −/− mice (B6;129P2-Ptgs1^{tm1Unc}) were produced on a mixed B6;129P2 background by mating COX-1 −/− males to COX-1 +/− females on a rotational breeding scheme. Wild-type mice of the COX-1 line were produced by mating COX-1 +/+ males to COX-1 +/+ females. The COX-2 −/− (B6;129P2-Ptgs2^{tm1Smi}) and COX-2 +/+ mice were similarly produced on a mixed B6;129P2 background. Upon arrival at St. Joseph's Hospital (Phoenix, AZ), the mice were 9–10 wk old and had a body mass of 25–30 g. The mice were initially housed two per cage; after surgery, they were housed individually. Standard mice chow (Purina, Richmond, IA) and tap water were available ad libitum. The room was maintained on a 12:12-h light-dark cycle (lights on at 7:00 AM) and at an ambient temperature of 27°C. Each mouse was systematically adapted (7 daily training sessions, 4 h each) to spend time inside a Plexiglas enclosure (length, 15 cm; width, 15 cm; height, 25 cm). The enclosures did not limit the animals' movement and were used later in the experiments. All experiments were started between 8:00 AM and 9:00 AM. Each mouse was used in only one experiment and euthanized with pentobarbital sodium (20 mg/kg iv) immediately thereafter. The protocols were approved by the St. Joseph's Hospital Animal Care and Use Committee.

Surgery. Two surgical procedures were performed: jugular vein catheterization and implantation of a temperature transmitter in the peritoneal cavity. These procedures were performed under ketamine-xylazine-acepromazine (42.0, 4.8, and 0.6 mg/kg, respectively, ip) anesthesia and antibiotic (enrofloxacin, 3.8 mg/kg sc) protection. A silicone catheter was passed into the superior vena cava through the jugular vein, and the free end of the catheter was exteriorized at the nape. A temperature transmitter (series 4000 E-Mitter; Mini Mitter, Bend, OR) was implanted via a midline laparotomy. For details on surgical procedures, see the companion paper (36). On *day 1* postsurgery, the jugular catheter was flushed with heparinized (50 U/ml) pyrogen-free saline. The mice tolerated the surgical procedures well; they showed only a minor (5–10%) loss of body mass on *day 1* and regained mass on *day 2*. The experiments were performed on *day 3*.

Experimental setup and protocol. The abdominal temperature, a measure of T_b, was recorded by telemetry at 2-min intervals. Telemetry receivers (model ER-4000; Mini Mitter) were positioned inside a climatic chamber (model 3940; Forma Scientific, Marietta, OH) and connected to a computer. The home cage of each mouse was placed on top of a receiver, a Plexiglas enclosure was placed inside the cage, and the mouse was left in the enclosure. The jugular catheter was extended with a length of polyethylene-50 (PE-50) tubing prefilled either with a combination of LPS suspension (25–30 μl at the mouse end of the extension) and saline (in the rest of the extension) or with saline alone. When the combination of LPS suspension and saline was used, a small (0.5 μl) air bubble separated the two. The extension was passed through a hanger on the top of the enclosure and a wall port of the climatic chamber. The extension was connected to a syringe filled with saline. The chamber was set to an ambient temperature of 31°C, which is neutral for mice in this experimental setup (36). The mice were allowed to habituate to these experimental conditions for ~2 h. Thereafter, they were injected intravenously with either *Escherichia coli* 0111:B4 LPS (Sigma, St. Louis, MO) or saline. Two doses of LPS were chosen based on our recent study (36): 1 μg/kg (10^0 μg/kg; caused a monophasic fever in C57BL/6 mice under the same experimental conditions) and 56 μg/kg ($10^{1.75}$ μg/kg; caused a polyphasic fever). To administer LPS, the LPS suspension in the mouse end of the catheter extension was flushed into the mouse's circulation by injecting saline (0.3 ml) from the syringe end of the extension. Hence, the procedure was executed from outside the chamber without touching the animal or causing pain or stress.

Data analysis. T_b data were compared across genotypes, treatments, and time points by a three-way ANOVA using Statistica AX'99 (StatSoft, Tulsa, OK). F and P values are reported for selected time intervals. Intervals corresponding to the duration of monophasic fever and durations of all phases of polyphasic fever were selected for each genotype based on preliminary experiments. A similar approach has been used in previous studies (9, 44). The compared values were considered significantly different at $P < 5.0 \times 10^{-2}$. The results are reported as means ± SE.

RESULTS

In all of the mice (regardless of the genotype), intravenous saline caused no significant change in T_b (Figs. 1 and 2). In the wild-type mice (COX-1 +/+ and COX-2 +/+), the low dose of LPS (1 μg/kg) caused a single, monophasic T_b rise, but the dynamics of the response differed. In the COX-1 +/+ mice, T_b started to increase at ~60 min, peaked at ~90 min, and returned to baseline at ~130 min postinjection (Fig. 1). In the COX-2 +/+ mice, the response peaked at the same time (~90 min), but started earlier (~30 min) and ended later (~210 min) (Fig. 2). Compared with saline, the low dose of LPS produced significant effects in both the COX-1 +/+ (60–130 min, $F_{1,96} = 1.3 \times 10^1$, $P = 3.8 \times 10^{-4}$) and COX-2 +/+ (30–210 min, $F_{1,168} = 7.6 \times 10^1$, $P = 1.0 \times 10^{-7}$) mice. To the moderate dose of LPS (56 μg/kg), the COX-1 +/+ and COX-2 +/+ mice responded with larger T_b rises, and the responses of the two wild-type lines had different dynamics. In the COX-1 +/+ mice, T_b started to increase at ~20 min and returned to baseline at ~220 min; the response consisted of the first (20–80 min) and second (80–160 min) febrile phases but did not have a clear third phase (Fig. 1). In the COX-2 +/+ mice, T_b also started to increase at ~20 min, but it had not returned to baseline until the end of experiment (270 min); this response consisted of three febrile phases (20–100, 100–150, and 150–270 min, respectively; Fig. 2). Compared with saline, the moderate dose of LPS produced significant effects in both the COX-1 +/+ (20–160 min, $F_{1,225} = 1.8 \times 10^2$, $P = 1.0 \times 10^{-7}$) and COX-2 +/+ (20–270 min, $F_{1,389} = 2.4 \times 10^2$, $P = 1.0 \times 10^{-7}$) mice. In general, the febrile responses of the COX-2 +/+ mice resembled those seen in C57BL/6 mice (36), whereas the febrile responses of COX-1 +/+ mice were somewhat different. Several other responses (e.g., arachidonic acid-induced ear swelling and ANG II-induced hypertension) have also been shown to differ in their timing and magnitude between the COX-1 +/+ and COX-2 +/+ lines (21, 23, 29).

Compared with the corresponding wild-type controls, neither monophasic fever nor the first phase of polyphasic fever was attenuated in the COX-1 −/− mice (Fig. 1). In fact, their fever responses were accelerated (monophasic fever) or enhanced (polyphasic fever). In contrast to the effects of COX-1 deletion, the effects of COX-2 deletion were drastic (Fig. 2).

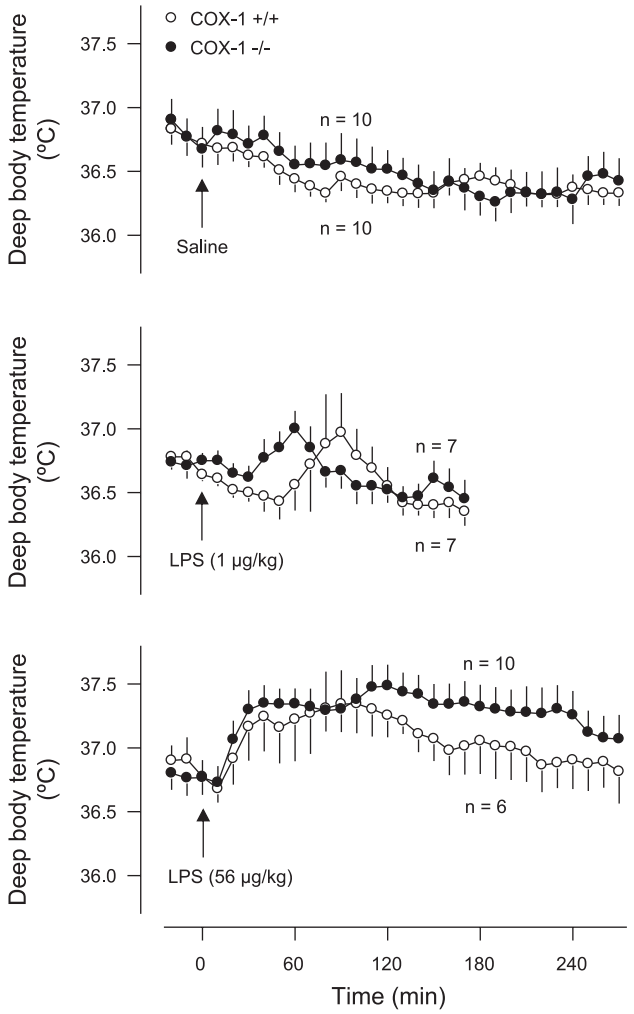

Fig. 1. Effects of an intravenous injection (arrow) of LPS (doses indicated) or saline on the deep body temperature of cyclooxygenase (COX)-1 +/+ (sufficient) and COX-1 −/− (deficient) mice at a neutral ambient temperature (31°C); *n*, number of mice.

Both monophasic fever ($F_{1,180} = 1.9 \times 10^2$, $P = 1.0 \times 10^{-7}$; intergenotype difference) and the first phase of polyphasic fever ($F_{1,108} = 1.9 \times 10^2$, $P = 1.0 \times 10^{-7}$) were absent in COX-2 −/− mice. The second and third phases of polyphasic fever were also missing in these mice ($F_{1,204} = 1.9 \times 10^2$, $P = 1.0 \times 10^{-7}$).

DISCUSSION

An involvement of COX, whether COX-1 or COX-2, in monophasic fever and the first phase of polyphasic fever is evident from the reported ability of nonselective (or relatively nonselective) COX inhibitors to attenuate both responses and the associated bursts in PGE_2 synthesis (24, 35, 37, 39, 40, 42). In the present study, we examined which COX isoform mediates each of these responses. We have found that both monophasic fever and the first phase of polyphasic fever do not occur in COX-2 −/− mice, thus indisputably demonstrating a critical role for COX-2 in both responses. An involvement of COX-2 in the mediation of the first phase of LPS fever has been previously suggested based on the fact that this enzyme is transcriptionally upregulated in the liver and lung already at the onset of the first febrile phase (15). However, transcriptional upregulation is not the only mechanism for activation of COX-2. This isoform can also be activated by the rapid reaction of tyrosine phosphorylation (27). In those organs where COX-2 is constitutively expressed (e.g., the lung and brain; see Refs. 17, 26, 46), such posttranscriptional activation may represent a mechanism for fast activation of this isoform. Hence, it remains to be investigated whether it is the inducible or the constitutive COX-2 that triggers the febrile response.

The demonstrated inability of COX-2 −/− mice to develop monophasic fever and the first phase of polyphasic fever also sheds light on the potential involvement of COX-1 in these responses. COX-2 −/− mice exhibit a compensatory, transcriptional upregulation of COX-1 (1, 18, 47). The fact that they develop no fever despite such upregulation suggests that COX-1 is not a critical mediator of the febrile responses studied. This suggestion is in line with the present results obtained in COX-1 −/− mice. The latter knockout mice showed no attenuation of either monophasic fever or any phase of polyphasic fever. In fact, their monophasic fever was accelerated, and their polyphasic fever was enhanced. Although negative results in genetically modified animals are often

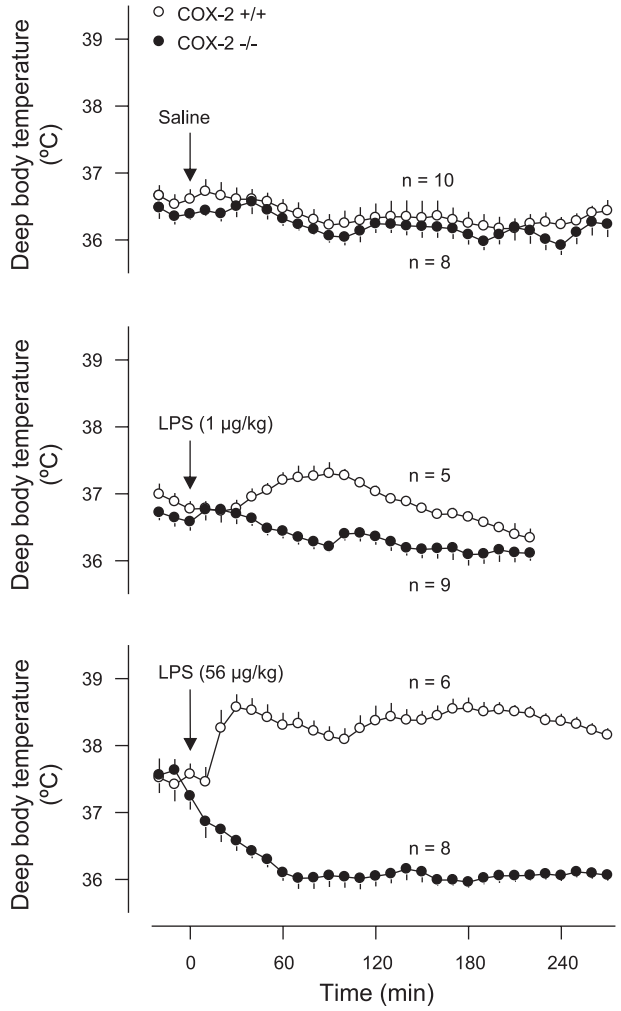

Fig. 2. Effects of an intravenous injection (arrow) of LPS (doses indicated) or saline on the deep body temperature of COX-2 +/+ and COX-2 −/− mice at a neutral ambient temperature (31°C); *n*, number of mice.

difficult to interpret due to potential compensatory mechanisms, the obtained combination of the negative results in COX-1 −/− mice (fever not attenuated) with the positive results in COX-2 −/− mice (fever abolished) leaves little room for any explanation other than that the responses of interest are mediated by one or more products of COX-2 and do not require any product of COX-1. That COX-1 is uninvolved in the mediation of the first febrile phase has also been shown in experiments with the highly selective COX-1 inhibitor, SC-560 (42).

Although SC-560 inhibits COX-1, it is unknown whether it also inhibits the splice variant of COX retaining intron 1 (COX-1V$_1$), also known as COX-1b or COX-3 (5, 7). COX-1b has been proposed (5) to be a target for the action of acetaminophen (paracetomol; a widely used antipyretic drug), and involvement of COX-1b in a variety of inflammatory diseases is currently under investigation (6, 10). In contrast to the unknown effect of SC-560 on COX-1b, the effects of the genetic tools used in the present study are known: knocking out the COX-1 gene eliminates the COX-1b enzyme, and knocking out the COX-2 gene leads to COX-1b overexpression (1). Hence, the combination of negative results in COX-1 −/− mice (fever not attenuated) and positive results in COX-2 −/− mice (fever abolished) also indicates that COX-1b is involved in the genesis of neither monophasic fever nor the first phase of polyphasic LPS fever. Such uninvolvement would be in line with several recent studies showing that COX-1b is not a target for acetaminophen (13, 20), and that this protein is not induced by acute inflammatory stimuli in rodents (19, 20, 38). Furthermore, it appears that the mouse (38), rat (41), and human (11) COX-1b mRNAs are shifted out of frame by intron 1 retention and are expected to yield an inactive enzyme. That the rat COX-1b protein does not have COX activity has recently been confirmed (41).

In summary, the present study identifies a new, critical role for COX-2 in the mediation of the earliest responses to intravenous LPS: monophasic fever and the first phase of polyphasic fever. It also suggests that no product of the COX-1 gene is essential for these responses.

ACKNOWLEDGMENTS

We thank Dr. V. F. Turek, M. C. Almeida, J. J. Burmeister, and J. L. Roberts for editing the manuscript.

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GRANTS

The study was funded in part by National Institute of Neurological Disorders and Stroke Grant R01-NS-41233 (to A. A. Romanovsky).

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