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Expression of genes controlling transport and catabolism of prostaglandin E_2 in lipopolysaccharide fever

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Ivanov, Andrei I., Adrienne C. Scheck, and Andrej A. Romanovsky. Expression of genes controlling transport and catabolism of prostaglandin E_2 in lipopolysaccharide fever. *Am J Physiol Regul Integr Comp Physiol* 284: R698–R706, 2003. First published October 24, 2002; 10.1152/ajpregu.00570.2002.—Prostaglandin (PG) E_2 is a principal downstream mediator of fever and other symptoms of systemic inflammation. Its inactivation occurs in peripheral tissues, primarily the lungs and liver, via carrier-mediated cellular uptake and enzymatic oxidation. We hypothesized that inactivation of PGE_2 is suppressed during LPS fever and that transcriptional downregulation of PGE_2 carriers and catabolizing enzymes contributes to this suppression. Fever was induced in inbred Wistar-Kyoto rats by intravenous LPS (50 μg/kg); the controls received saline. Samples of the liver, lungs, and hypothalamus were harvested 0, 0.5, 1.5, and 5 h postinjection. The expression of the two principal transmembrane PGE_2 carriers (PG transporter and multispecific organic anion transporter) and the two key PGE_2-inactivating enzymes [15-hydroxy-PG dehydrogenase (15-PGDH) and carbonyl reductase] was quantified by RT-PCR. All four genes of interest were downregulated in peripheral tissues (but not the brain) during fever. Most remarkably, the expression of hepatic 15-PGDH was decreased 26-fold 5 h post-LPS, whereas expression of pulmonary 15-PGDH was downregulated (as much as 18-fold) throughout the entire febrile course. The transcriptional downregulation of several proteins involved in PGE_2 inactivation, first reported here, is an unrecognized mechanism of systemic inflammation. By increasing the blood-brain gradient of PGE_2, this mechanism likely facilitates penetration of PGE_2 into the brain and prevents its elimination from the brain.

systemic inflammation; multispecific organic anion transporter; prostaglandin transporter; 15-hydroxyprostaglandin dehydrogenase; carbonyl reductase

ON A SYSTEMIC INFLAMMATORY challenge (e.g., with bacterial LPS or pyrogenic cytokines, such as IL-1β and TNF-α), the brain launches a powerful defense response: fever (29, 51). Fever is mediated by prostaglandin (PG) E_2 (8). Indeed, pharmacological blockade (42, 58) or genetic abrogation (30, 43, 65) of PG synthesis or PGE receptors attenuates this response. Fever-associated production of PGE_2 occurs via transcriptional upregulation of several enzymes within the PGE_2-synthesizing cascade, most markedly secretory phospholipase A_2 group IIA, cyclooxygenase (COX)-2, and microsomal PGE synthase (17, 26, 34, 68). Pyrogenic PGE_2 is generally thought to be produced in the hypothalamus, the brain's "febrigenic center" (8). Yet, a contribution of peripherally originated PGE_2 to the febrile response has also been proposed (12) and confirmed by several lines of studies (13, 16, 47). Importantly, the PGE_2-synthesizing enzymes are induced during fever not only in the brain but also in the LPS-processing organs: the liver and lungs (26, 34). As a result, an increased blood level of PGE_2 has been found in many (e.g., 13, 38, 52, 59, 60), although not all (32), studies. Such an increase is likely to contribute to febrile pathogenesis because the ability of intravenous or intra-arterial PGE to successfully reach the brain and cause fever has been shown in at least nine studies (for review, see Ref. 47).

The liver and lungs are also responsible for inactivation of PGs (45, 53), which, in addition to synthesis, is a rapid mechanism to change the blood level of these short-lived molecules. Inactivation of PGE_2 occurs intracellularly; it requires, first, carrier-mediated transport across the cell membrane and, second, intracellular catabolism (18, 57). Carrier-mediated transport is essential because PGs diffuse through membranes poorly (3, 57). In fact, transport is the rate-limiting step of PGE_2 inactivation, at least in the lungs (4). The major carriers of PGE_2 are PG transporter (PGT; also known as matrin) and multispecific organic anion transporter (MOAT) (41, 57). Intracellular catabolism of PGE_2 consists of several subsequent reactions, of which the first reaction, enzymatic oxidation of the 15-hydroxyl group, is crucial because it leads to the loss of biological activities (18). This reaction is catalyzed by 15-hydroxy-PG dehydrogenase (15-PGDH; formerly known as 15-PGDH type I) and carbonyl reductase (CR; formerly, 15-PGDH type II) (18, 21). CR also possesses 9-keto-reductase activity and thus inactivates PGE_2 by converting it to $PGF_{2\alpha}$ (63), a much less potent inductor of fever and other inflammatory

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symptoms (36, 62). Physiological importance of enzymatic oxidation of PGE_2 is supported by the fact that both genetic deletion of 15-PGDH (11) and its transcriptional downregulation (e.g., as a result of thermal injury; see Ref. 22) lead to an increase in the blood and/or tissue levels of PGE_2.

It is noteworthy that PGE_2 is not catabolized in the brain of adult mammals (40). To be inactivated, brain PGE_2 has first to escape the brain tissue and enter the blood, which supposedly happens in the choroid plexus (15). Next, PGE_2 has to be delivered with the circulation to the lungs and liver, where it is transported inside the cells and oxidized as described above. A high transchoroid (brain to blood) PGE_2 gradient has been shown to facilitate the escape of PGE_2 from the brain, whereas a low gradient impedes such an escape (5). Hence, by changing the blood concentration of PGE_2, the processes of transport and catabolism of PGE_2 in peripheral organs can affect the level of PGE_2 not only in peripheral tissues, but also in the brain. It is tempting, therefore, to speculate that carrier-mediated transport across the cell membrane and intracellular catabolism of PGE_2 in the liver and lungs can be used by the body to regulate the febrile and other physiological responses driven by peripheral and/or central PGE_2.

However, little is known about regulation of either transmembrane transport or enzymatic oxidation of PGE_2 in fever and systemic inflammation. One study showed that PGE_2 transport was inhibited in the rabbit uvea by topical application of LPS (2), whereas another study failed to detect any effect of IL-1β on PGT expression in human endothelial cells in vitro (64). Administration of IL-1β or TNF-α in vitro (37, 46) and shock-inducing doses of LPS in vivo (6, 23, 39) were shown to inhibit the catabolism of PGE_1 and PGE_2 and expression of 15-PGDH. No studies on fever or inflammation have focused on the expressional regulation of MOAT or CR.

We hypothesized that the febrile response, even to mild doses of LPS, is accompanied by transcriptional downregulation of proteins involved in inactivation of PGE_2. To test this hypothesis, we measured mRNA expression of two principal carriers (PGT and MOAT) and two principal catabolizing enzymes (15-PGDH and CR) of PGE_2 in the major PG-inactivating organs (the liver and lungs) and in the brain's febrigenic center (hypothalamus) by quantitative RT-PCR. A rat model of intravenous LPS-induced fever was used. It is noteworthy that the fever response to mild doses of intravenous LPS is polyphasic, and three distinct febrile phases (*phases I*, *II*, and *III*) have been identified in rats and mice (43, 49, 50). Moreover, different febrile phases are characterized by different patterns of transcriptional regulation of PGE_2-synthesizing enzymes (26) and may involve different PGE receptors (43). All three phases were studied in the present work. Preliminary results of this study are published elsewhere (27).

MATERIALS AND METHODS

Animals. Fifty-seven 2-mo-old male inbred Wistar-Kyoto rats (Harlan Sprague Dawley, Indianapolis, IN) were used in this study. All animals were initially housed three per standard "shoebox"; after surgery, they were caged individually. The cages were ventilated by a Smart Bio-Pack system with Thermo-Pak temperature control module (Allentown Caging Equipment, Allentown, NJ). This system supplied the cages with warmed (27°C) air. Thermally neutral environment inside the cages was verified by the absence of tail skin vasoconstriction and vasodilation (48). The room was on a light-dark cycle of 12:12 h (lights on from 7:00 AM to 7:00 PM). Food (Teklad Rodent Diet "W" 8604, Harlan Teklad, Madison, WI) and water were available ad libitum. The cage space was enriched with artificial "rat holes" (cylindrical confiners made of stainless steel wire). In addition to spending time in the confiners voluntarily, the rats were systematically habituated to them (5 training sessions, 4–5 h each). The same confiners were used later in experiments. Well-adapted, confined rats exhibit neither a stress fever nor any other signs of stress (49). Each animal was subjected to chronic jugular catheterization under intraperitoneal ketamine-xylazine-acepromazine (55.6, 5.5, and 1.1 mg/kg, respectively) anesthesia, as described elsewhere (26, 49). A silicone catheter was passed into the vena cava superior through the jugular vein, and the free end of the catheter was exteriorized at the nape. On *day 1* postsurgery, the catheter was flushed with heparinized (50 IU/ml) saline. On *day 3*, the animal was used in an experiment. All experiments started between 8:00 and 9:00 AM. The protocols have been approved by the Institutional Animal Care and Use Committee.

Experiment 1. In this experiment, the dynamics of the febrile response to LPS were determined by measuring colonic temperature (T_c), an index of body core temperature. Fifteen rats were placed in their confiners, and copper-constantan thermocouples were inserted 9 cm beyond the anus. The thermocouples were connected to a data logger (model AI-24, Dianachart, Rockaway, NJ) and personal computer. The animals were transferred to a climatic chamber (Forma Scientific, Marietta, OH) set to 30°C (the midpoint of the thermoneutral zone for Wistar rats; see Ref. 48) and 50% relative humidity. The exteriorized portions of the jugular catheters were pulled through a wall port and connected to syringes filled with either *Escherichia coli* 0111:B4 LPS (Sigma Chemical, St. Louis, MO; 50 μg/ml) or saline. After a 2-h stabilization period, the animals were injected with either LPS (50 μg/kg; 7 rats) or saline (1 ml/kg; 8 rats). Their T_c was measured from 1 h before to 7 h after the injection.

The ultimate goal of *experiment 1* was to identify three time points (1 for each febrile phase) for tissue harvesting in *experiment 2*. It is not T_c per se, but rather its velocity (1st derivative), that is proportional to the rate of change of the total heat content in the body (the sum of heat loss and heat production), which is determined by the activity of thermoregulatory effectors. Therefore, local maxima of T_c velocity likely correspond to peaks of biochemical changes that drive thermoeffectors. For this reason, the times for tissue harvesting were identified as the three local maxima of the T_c velocity at the three febrile phases. The T_c curves were averaged across the subjects, the resultant curve was smoothed, and its first derivative was computed using Origin 6.0 (Microcal Software, Northampton, MA). The three points were identified as ~0.5 h (*phase I*), 1.5 h (*phase II*), and 5 h post-LPS (*phase III*).

Experiment 2. Seven groups of rats (6 animals in each group) were prepared as for *experiment 1*, except that no

thermocouples were inserted. Three groups received LPS (50 μg/kg); their tissues were harvested 0.5, 1.5, or 5 h postinjection. Another three groups received saline; their tissues were harvested at the same time points. The remaining group received no injection and served as an untreated control; their tissues were harvested at the point corresponding to the time of LPS or saline injection in the other six groups (*time 0*). This design allowed us to express the results obtained in LPS- and saline-treated rats relative to the untreated controls and to thus account for potential circadian dynamics in PG synthesis (54) and possibly PG inactivation in afebrile rats.

For tissue harvesting, each rat was anesthetized with intravenous ketamine-xylazine-acepromazine (5.6, 0.6, and 0.1 mg/kg, respectively). To immediately stop RNA degradation, the anesthetized animal was perfused through the left ventricle (right atrium cut) with 30 ml of saline followed by 30 ml of an RNA-preserving solution, RNAlater (Ambion, Austin, TX), diluted twofold with saline. Samples of the liver (~300 mg) and right lung (~150 mg) were collected rapidly and snap-frozen in liquid nitrogen. The anesthetized animal was decapitated, its brain was removed, and the entire hypothalamus (~80 mg) was dissected and frozen. All samples were stored at −80°C.

RNA isolation and RT-PCR. Total RNA was isolated from the tissue samples using Qiagen RNAeasy kits (Qiagen, Valencia, CA) and treated with DNase I (Ambion), as described previously (26). Its purity (260 nm:280 nm absorption ratio > 1.9) and integrity (presence of 2 sharp 28S and 18S rRNA electrophoretic bands) were verified, and the amount of the isolated RNA was quantified by absorption at 260 nm. Total RNA was reverse transcribed to cDNA by random hexamer priming using GeneAmp RNA PCR kit (Applied Biosystems, Foster City, CA) and SuperScript II RT (Invitrogen, Carlsbad, CA). All liver and lung RNA samples were 2 μg each; all hypothalamic samples were 1 μg each; the reaction volume was 20 μl. For quantitative real-time PCR, a LightCycler (Roche Molecular Biochemicals, Indianapolis, IN) was used. The concentration of double-stranded DNA amplicon was monitored by SYBR Green I fluorescence. Primers for the genes of interest and glyceraldehyde-3-phosphate dehydrogenase (GAPDH), a housekeeping gene, were found in the literature or designed based on the rat sequences deposited in GenBank (Table 1). Sample preparation and detailed protocols for PCR are described elsewhere (26). Specificity of amplification was verified by running agarose gel electrophoresis of each amplicon and obtaining a single band of the expected size (Table 1). For each gene of interest in each tissue, the obtained PCR products were independently identified by sequencing on an ABI 377 automated DNA sequencer (Applied Biosystems, Foster City, CA) as described earlier (26). From each tissue, 16 randomly selected RNA samples were run together in each RT-PCR: two from each of the three LPS-treated groups; two from each of the three saline-treated groups; two from the untreated group; and two additional samples (1 from a saline- and the other from LPS-treated group) with no RT added (so-called "−RT" controls). Thus, for each tissue, three independent runs of RT-PCR were performed in duplicate.

Data processing and analysis. The relative expression R of each gene of interest was calculated as follows

$$R_{i,t} = 2^{(N_{h,t} - N_{h,c}) - (N_{i,t} - N_{i,c})} \quad (1)$$

where N is the threshold cycle number, i.e., the number of the amplification cycle in which the fluorescence of a given sample becomes significantly different from the baseline signal. The indexes i and h refer to the gene of interest and housekeeping gene (GAPDH), respectively. The index t refers to a sample from a treated (with either LPS or saline) animal. The indexes c refer to the samples from the two untreated controls run in the same RT-PCR (the variables $N_{i,c}$ and $N_{h,c}$ were the means for the 2 controls in $\log_2$ scale). *Equation 1* is based on the inverse proportionality between N and $\log_2 C$, where C is the initial template concentration in the PCR sample (9). Hence the physical meaning of $R_{i,t}$ is the concentration of mRNA of interest in a sample from a treated animal divided by the concentration of the same message in the simultaneously run samples from untreated controls, where each concentration is normalized for the concentration of GAPDH message in the same sample.

The T_c responses and the relative expression data for each gene in each tissue were compared across treatments (LPS vs. saline) and time points (*phases I*, *II*, and *III*) by two-way ANOVA followed by Newman-Keuls post hoc test using Statistica AX'99 (StatSoft, Tulsa, OK). All data are presented as means ± SE.

RESULTS

As in the past (26), the inbred Wistar-Kyoto rats responded to intravenous LPS (50 μg/kg) with a triphasic fever ($P < 1.1 \times 10^{-6}$), whereas no fever developed in the saline-treated animals (Fig. 1*A*). The time points for tissue harvesting were identified as the three local maxima of the T_c velocity occurring at ~0.5 h (*phase I*), 1.5 h (*phase II*), and 5 h (*phase III*) post-LPS (Fig. 1*B*).

Table 1. *Primers used for quantitative RT-PCR*

Gene Name (GenBank Entry No.)	Primers	Reference No.	Amplicon Size, bp	Annealing Temperature, °C
PGT (NM_022667)	F: 5′-gagcagtctcaccacaatcg R: 5′-ggctcggcaaagtcatccac	28	424	62
MOAT (AF169409)	F: 5′-tctcagaaccataccgctacg R: 5′-aacaggattcccaggtagagg	this study	276	58
15-PGDH (NM_024390)	F: 5′-atgcacgtgaacggcaaagtg R: 5′-ttcactcctgcgttgttgacc	this study	293	60
CR (NM_019170)	F: 5′-gctggcccaatagtgcatatgg R: 5′-tggctttcaccaagtcaggatag	this study	387	62
GAPDH (X02231)	F: 5′-agacagccgcatcttcttgt R: 5′-ccacagtcttctgagtggca	10	587	58

F, forward; R, reverse; PGT, PG transporter; MOAT, multispecific organic anion transporter; 15-PGDH, 15-hydroxy-PG dehydrogenase; CR, carbonyl reductase; GAPDH, glyceraldehyde 3-phosphate dehydrogenase.

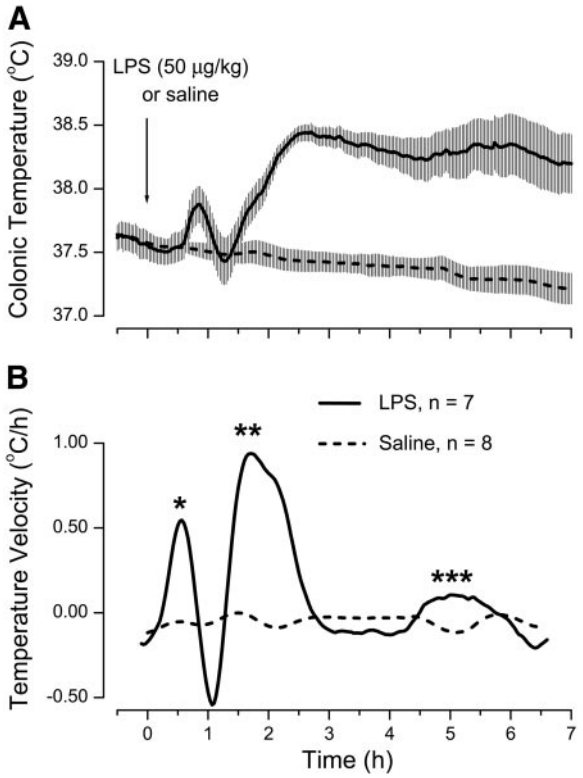

Fig. 1. Thermal response of inbred Wistar-Kyoto rats to an intravenous injection of LPS or saline at *time 0* (arrow). *A*: the colonic temperature. *B*: its velocity (1st derivative). The 3 local maxima of the velocity (marked as *, **, and ***) determine time points for tissue harvesting at febrile *phases I* (0.5 h postinjection), *II* (1.5 h), and *III* (5 h), respectively.

In all organs studied (liver, lungs, and hypothalamus), transcripts of all genes of interest (PGT, MOAT, 15-PGDH, and CR) and the housekeeping gene (GAPDH) were amplified as single products (Fig. 2). No amplification was detected in −RT controls (not shown). No significant changes in the expression of GAPDH were found (Fig. 2). For no gene and in no organ was the tissue concentration of the transcript changed in the saline-treated animals at any time point (see Fig. 3 for PGT, Fig. 4. for MOAT, Fig. 5 for 15-PGDH, and Fig. 6 for CR), thus suggesting that no circadian rhythms in the expression of these genes can be found over the short time span studied, i.e., from ~10:30 AM (time of intravenous injection) to ~3:30 PM (5 h postinjection).

The LPS-treated animals showed profound changes in the mRNA concentration of all genes of interest in the liver and lungs. Both transporters (PGT and MOAT) were downregulated at febrile *phase III*. At that phase, the concentration of PGT mRNA was decreased ~5-fold ($P < 1.9 \times 10^{-4}$) in the liver and 3-fold ($P < 7.4 \times 10^{-3}$) in the lungs (Fig. 3), and the tissue concentration of MOAT mRNA was decreased ~3-fold ($P = 4.1 \times 10^{-4}$) in the liver and 2-fold ($P < 5.0 \times 10^{-2}$) in the lungs (Fig. 4). Both dehydrogenases (15-PGDH and CR) were also downregulated by LPS in the peripheral tissues. The concentration of 15-PGDH transcript in the liver was decreased only at *phase III* (~26-fold, $P < 2.2 \times 10^{-3}$), whereas pulmonary transcription was significantly downregulated at all phases: ~2-fold at *phase I* ($P < 2.9 \times 10^{-2}$), 5-fold at *phase II* ($P < 2.3 \times 10^{-3}$), and 18-fold at *phase III* ($P < 1.5 \times 10^{-2}$) (Fig. 5). The transcript level for CR in the liver was decreased ~5-fold ($P = 2.1 \times 10^{-4}$) at *phase III* (and showed a tendency to decrease at *phase II*). In the lungs, transcription of CR was downregulated ~3-fold at both *phases II* ($P < 4.2 \times 10^{-2}$) and *III* ($P < 2.5 \times 10^{-2}$) (Fig. 6). In the hypothalamus, the expression of

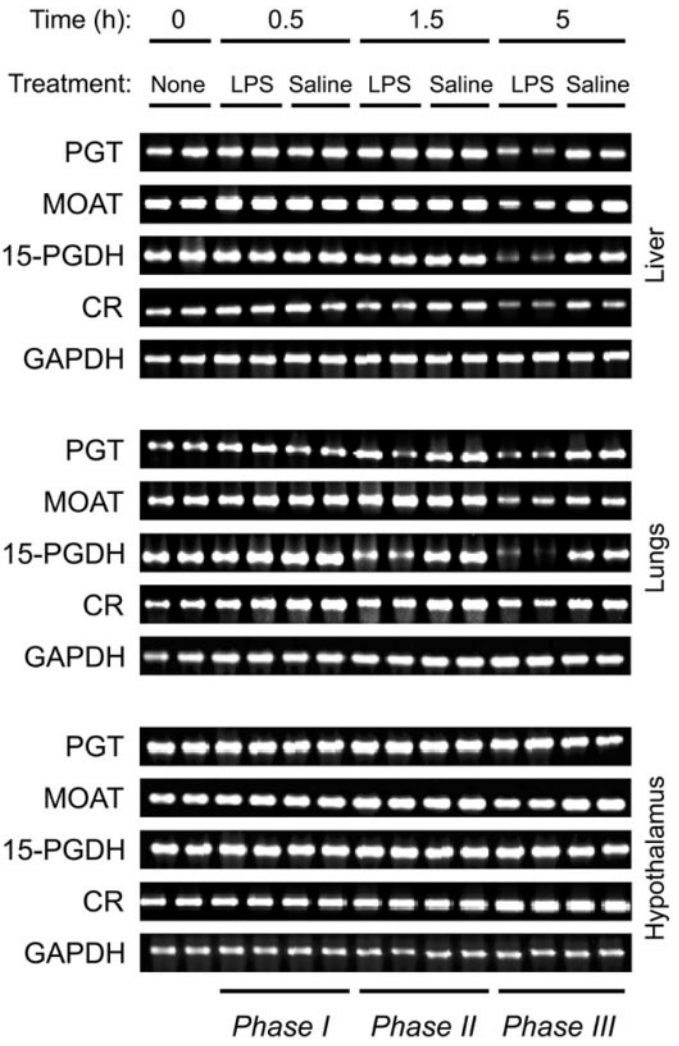

Fig. 2. Expression of PGE_2 carriers [PG transporter (PGT) and multispecific organic anion transporter (MOAT)], PGE_2-inactivating enzymes [15-hydroxy-PG dehydrogenase (15-PGDH) and carbonyl reductase (CR)], and a housekeeping gene (GAPDH) in rat liver, lungs, and hypothalamus at different times after an intravenous injection of LPS (50 μg/kg) or saline. For each gene in each tissue, 14 representative samples are shown: 2 from untreated controls; 2 from LPS- and 2 from saline-treated rats killed 0.5 h postinjection (*phase I*); 2 from LPS- and 2 from saline-treated rats killed 1.5 h postinjection (*phase II*); and 2 from LPS- and 2 from saline-treated rats killed 5 h postinjection (*phase III*). The LightCycler PCR reactions were stopped at the exponential phase of amplification. Amplicons were separated in a 1.5% agarose gel and visualized by SYBR Gold nucleic acid stain.

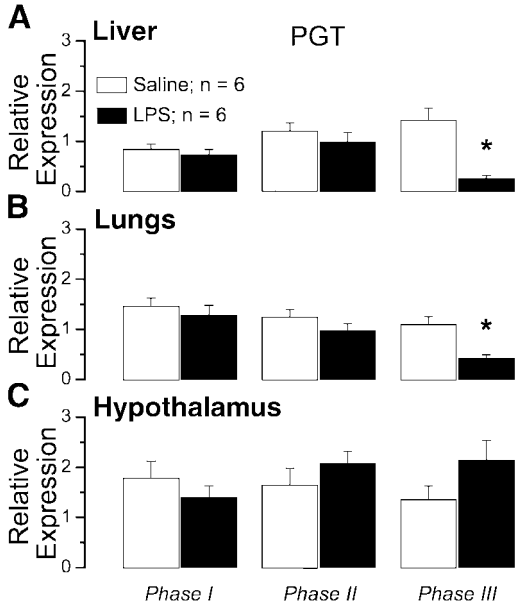

Fig. 3. The relative expression of PGT gene in the liver (*A*), lungs (*B*), and hypothalamus (*C*) at *phases I*, *II*, and *III* of LPS (50 μg/kg iv) fever and at the corresponding time points after an injection of saline. Each datum is a ratio, where the numerator is the concentration of PGT mRNA in a tissue sample collected at a given time point from a rat treated with either LPS or saline, and the denominator is the concentration of PGT mRNA in a sample harvested from an untreated control at *time 0*. To equalize cDNA content in different samples, the ratios were normalized for the concentration of GAPDH mRNA (see *Data processing and analysis*). $^*P < 0.05$.

no gene of interest was affected by intravenous LPS (Figs. 3–6).

DISCUSSION

Expression of PGE_2 transporters and dehydrogenases in fever: phenomenology. This study shows that the expression of two major transmembrane carriers of PGE_2 (PGT and MOAT) and its two inactivating enzymes (15-PGDH and CR) is downregulated during the febrile response to a mild dose of systemic LPS. Whereas transcriptional downregulation of pulmonary 15-PGDH in systemic endotoxemia has been reported (23), the dramatic (~26-fold) downregulation of this enzyme in the liver (Fig. 5) is a novel observation. Findings of downregulation of pulmonary and hepatic PGT (Fig. 3), MOAT (Fig. 4), and CR (Fig. 6) are also new; no data on the activity or expression of these enzymes in systemic inflammation or fever are available in the literature.

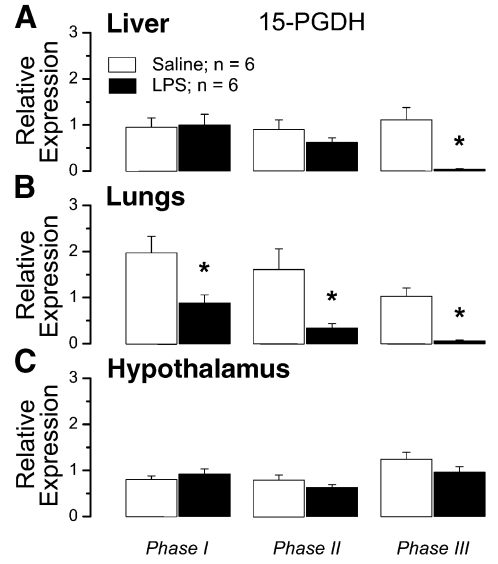

Fig. 5. The relative expression of 15-PGDH gene in the liver (*A*), lungs (*B*), and hypothalamus (*C*) at *phases I–III* of LPS fever and in saline-treated rats (see legend to Fig. 3). $^*P < 0.05$.

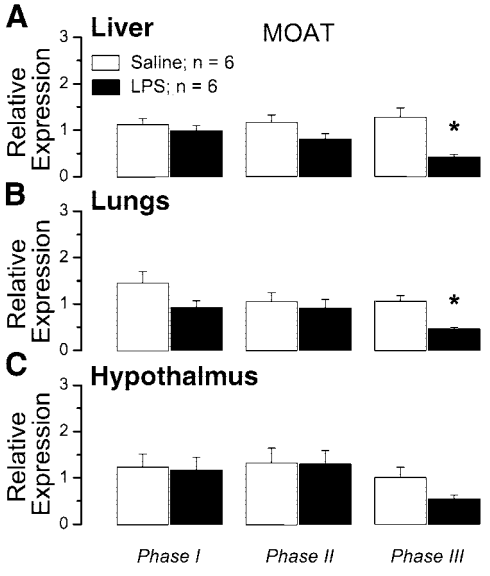

Fig. 4. The relative expression of MOAT gene in the liver (*A*), lungs (*B*), and hypothalamus (*C*) at *phases I–III* of LPS fever and in saline-treated rats (see legend to Fig. 3). $^*P < 0.05$.

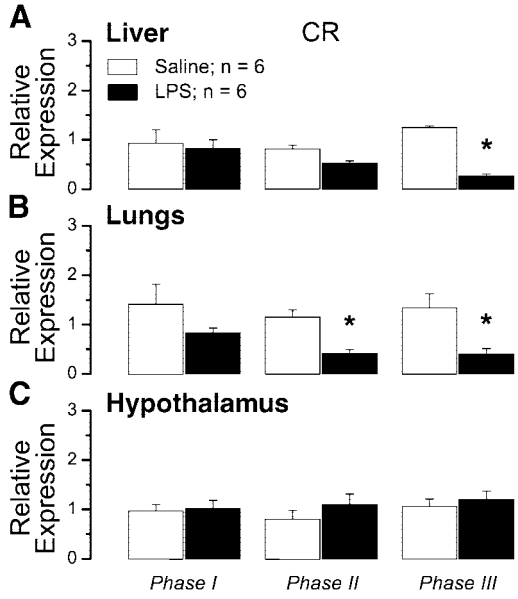

Fig. 6. The relative expression of CR gene in the liver (*A*), lungs (*B*), and hypothalamus (*C*) at *phases I–III* of LPS fever and in saline-treated rats (see legend to Fig. 3). $^*P < 0.05$.

Because transcription of no gene of interest in this study was "contaminated" by circadian rhythms, we summarized the results obtained by plotting the LPS/saline ratio for the expression of each gene in each tissue at each of the three febrile phases (Fig. 7). Figure 7 shows that transcriptional downregulation of PGE_2-transporting and -catabolizing proteins is robust (by up to 96%), coordinated in time (all genes of interest are maximally downregulated at *phase III*), and tissue specific. It occurs in the lungs and liver (the organs responsible for clearance of circulating PGs; see Refs. 45 and 53) but not in the brain (which, in adulthood, is devoid of substantial catabolism of PGE_2 and has little 15-PGDH or CR activity; see Refs. 40, 66). The observed tissue specificity may also reflect a poor permeability of the blood-brain barrier for LPS and endogenous mediators of its action.

Downregulation of PGE_2 catabolism in fever: physiological significance. The tissue specificity, high magnitude, and coordinated occurrence suggest that transcriptional downregulation of PGE_2-inactivating enzymes is likely to have physiological significance, especially during *phase III*. Teleologically, this proposition makes sense. PGE_2 is a rapidly acting, short-lived mediator. Its physiological effects (e.g., fever) have a latent period of a few minutes and duration of tens of minutes (61). Its half-life in the blood plasma is <60 s (25). Hence, the biological activity of PGE_2 is highly responsive to changes in its catabolism (24). It is also known that the half-life of the major dehydrogenase for PGE_2, 15-PGDH, is on the order of tens of minutes (7, 67). Moreover, it has been experimentally shown that changes at the mRNA level readily translate into corresponding changes at the protein level for PGE_2-inactivating enzymes (23, 67). This information allows us to speculate about the physiological significance of the drastic transcriptional downregulation observed in the present study even though our own work focused only on the expression of the PGE_2-inactivating enzymes at the mRNA level.

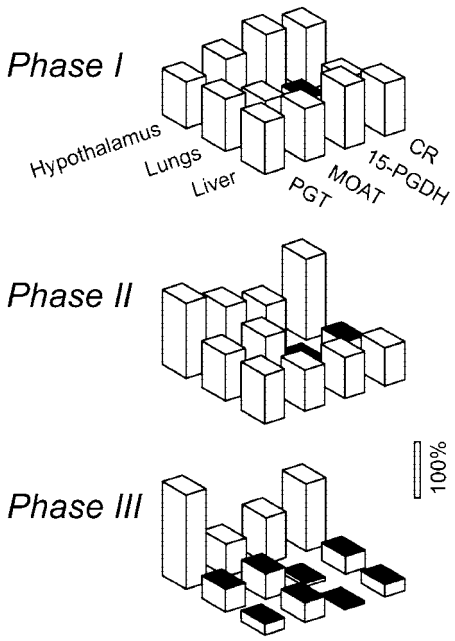

Fig. 7. *Phases I*, *II*, and *III* of LPS fever: expressional patterns of PGE_2 transporters and catabolizing enzymes. Each bar represents the relative expression of a given gene in a given tissue and at given time point (phase) of LPS fever as a percentage of the relative expression of the same gene in the same tissue and at the same time point after saline injection. Significant transcriptional downregulation of a given gene in a given tissue is indicated by black. Profound suppression of all genes of interest in peripheral tissues was found at *phase III*.

Not only do the present results make sense teleologically, but they also provide new explanations for two recently observed experimental phenomena. The first phenomenon was reported by Davidson et al. (13), who showed that intravenous administration of LPS, IL-1β, or TNF-α in rabbits readily facilitates PGE_2 entry from the peripheral circulation into the brain. The authors hypothesized that this phenomenon reflects a disruption of the blood-brain barrier in systemic inflammation. Our present results suggest that an accelerated influx of circulating PGE_2 into the brain during fever may occur even if the integrity of the barrier is uncompromised. The simultaneous, drastic transcriptional downregulation of four major PGE_2-inactivating enzymes in the liver and lungs is likely to increase the blood concentration of PGE_2 and, therefore, the blood-to-brain PGE_2 gradient. Yet, the expression of PGE_2 carriers in the hypothalamus per se remains unaltered during fever. In the presence of normally expressed PGE_2 carriers, the increased blood-brain PGE_2 gradient is likely to facilitate transport of circulating PGE_2 into the brain.

The second phenomenon was discovered in our recent study (26). Using the same model of the triphasic LPS fever in inbred Wistar-Kyoto rats, we found that transcription of COX-2, a key enzyme of PGE_2 synthesis, is significantly decreased during febrile *phase III* (compared with *phase II*) in all tissues studied. Because all three febrile phases, including *phase III*, are likely to be mediated by PGE_2 (43, 55), there should be mechanisms counteracting the transcriptional downregulation of COX-2 and ensuring a further increase in PGE_2 level during *phase III*. These mechanisms may include transcriptional upregulation of other PGE_2-synthesizing enzymes, viz. microsomal PGE synthase and several phospholipases A_2 (26). However, the efficiency of such compensatory upregulation is difficult to assess, because there is no consensus as to which of the reactions of the PGE_2-synthesizing cascade is rate limiting. A better compensatory mechanism, the one that will certainly work, can be proposed based on the present findings. It is the coordinated transcriptional downregulation of four major PGE_2-transporting and/or -inactivating proteins.

Transcriptional suppression of PGE_2-inactivating enzymes in fever: putative mechanisms. How the expression of PGE_2 transporters and catabolizing enzymes is regulated in fever or inflammation is largely unknown. Genomic sequences of human PGT (31), mouse 15-PGDH (35), and human CR (20) give an interesting hint: all three genes contain sequences for

Sp1 transcription factor. Hence, inactivation of constitutive transcription factor Sp1 by LPS and/or LPS-induced cytokines may be a common mechanism for transcriptional downregulation of PGT, 15-PGDH, and CR in fever. Indeed, LPS and TNF-α decrease DNA-binding activity of Sp1 and inhibit Sp1-mediated gene expression (14, 69). A notion that LPS-induced transcriptional downregulation of PGE_2-inactivating enzymes is mediated by cytokines is supported by the fact that both IL-1β and TNF-α decrease expression of 15-PGDH and inhibit PGE_2 catabolism in cultured human trophoblasts (37, 46).

A corticosteroid-dependent mechanism for transcriptional downregulation of PGE_2-inactivating enzymes has also been suggested based on the existence of glucocorticoid-responsive elements in the promoter of the 15-PGDH gene (35). Indeed, cortisol and dexamethasone downregulate transcription of 15-PGDH and/or inhibit its activity in human trophoblast cells in vitro (37, 44) and in rat kidney in vivo (19). Because LPS is a potent stimulus for corticosteroid release (33), transcriptional downregulation of 15-PGDH during LPS fever may be triggered by endogenous glucocorticoids. Such an action of glucocorticoids would agree with their "permissive" role in febrile pathogenesis (1, 56).

Perspectives

The febrile response to mild doses of LPS is accompanied by drastic (up to 26-fold) transcriptional downregulation of PGE_2 transporters (PGT and MOAT) and its catabolizing enzymes (15-PGDH and CR) in the lungs and liver, but not the brain. We speculate that this downregulation increases the blood-to-brain gradient of PGE_2 (decreases the brain-to-blood gradient) and is, therefore, likely to both facilitate penetration of PGE_2 into the central nervous system and prevent its elimination from the brain. This largely unrecognized mechanism may constitute a novel target for antipyretic/anti-inflammatory therapy.

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