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letters to the editor

Following are the abstracts of the articles discussed in the subsequent letter:

Imai-Matsumura, Kyoko, Kiyoshi Matsumura, Akira Terao, and Yasuyoshi Watanabe. Attenuated fever in pregnant rats is associated with blunted syntheses of brain cyclooxygenase-2 and PGE_2. *Am J Physiol Regul Integr Comp Physiol* 283: R1346–R1353, 2002.—Attenuation of fever occurs in pregnant animals. This study examined a hypothesis that brain production of PGE_2, the final mediator of fever, is suppressed in pregnant animals. Near-term pregnant rats and age-matched nonpregnant female rats were injected with lipopolysaccharide (100 μg/kg) intraperitoneally. Four hours later, colonic temperature was measured, their cerebrospinal fluid (CSF) was sampled for PGE_2 assay, and their brains were processed for immunohistochemistry of cyclooxygenase-2, an enzyme involved in PGE_2 biosynthesis. In the pregnant rats, lipopolysaccharide injection resulted in significantly smaller elevations in both colonic temperature and CSF-PGE_2 level than in nonpregnant rats. In the pregnant rats, lipopolysaccharide-induced cyclooxygenase-2 expression was blunted in terms of the number of positive cells. There was a significant correlation between PGE_2 level in CSF and the number of cyclooxygenase-2-positive endothelial cells. These results suggest that suppressed PGE_2 production in the brain is one cause for the attenuated fever response at near-term pregnancy and that this suppressed PGE_2 production is due to the suppressed induction of cyclooxygenase-2 in brain endothelial cells.

Mouihate, A., M.-S. Clerget-Froidevaux, K. Nakamura, M. Negishi, J. L. Wallace, and Q. J. Pittman. Suppression of fever at near term is associated with reduced COX-2 protein expression in rat hypothalamus. *Am J Physiol Regul Integr Comp Physiol* 283: R800–R805, 2002.—The fever response is blunted at near term. As the enzyme cyclooxygenase-2 (COX-2) plays a critical role in fever development, we measured its expression in rat hypothalamus during pregnancy and lactation. Western blot analysis revealed a 72-kDa COX-2-immunoreactive band in non-immune-challenged, pregnant rats at *day 15* of pregnancy. In contrast, it was almost undetectable at near term and at lactation *day 5*. COX-2 was significantly induced at the 15th day of pregnancy and at the 5th lactating day after intraperitoneal lipopolysaccharide (50 μg/kg). However, this COX-2 induction was significantly reduced at near term compared with values before and after term. The protein levels of the EP3 receptor in the hypothalamus, one of the prostaglandin E_2 (PGE_2) receptors suggested to be a key receptor for fever induction, were unaffected throughout the pregnancy and lactation in both non-immune-challenged and lipopolysaccharide-treated rats. These data suggest that suppression of fever at near term is associated with a significantly reduced induction of COX-2 by lipopolysaccharide, resulting in a reduced production of PGE_2. Altered expression of the EP3 receptor does not seem to be involved in this fever refractoriness at near term.

Near-term suppression of fever: inhibited synthesis or accelerated catabolism of prostaglandin E_2?

To the Editor: Recently, Mouihate et al. (12) and Imai-Matsumura et al. (6) proposed the intriguing hypothesis that decreased febrile responsiveness to LPS and cytokines at near term reflects the reduced expression of a PGE_2-synthesizing enzyme cyclooxygenase (COX)-2. The importance of this hypothesis was promptly recognized (14). The hypothesis is based on the observations that LPS-induced expression of COX-2 protein (12) and increase in the number of COX-2-positive cells (6) in the hypothalamus were both attenuated (<2-fold) in pregnant rats. Although profound pharmacological or genetic blockade of COX-2 does suppress fever, the febrile response is probably insensitive to small changes in COX-2 expression. Indeed, in vitro studies (for review, see Ref. 16) question a rate-limiting role for COX within the PGE_2-synthesizing cascade, whereas recent in vivo data (7, 8) demonstrate the lack of correlation between the tissue level of COX-2 (protein or mRNA) and either the concentration of PGE_2 or the height of fever. Consistent with these data, Imai-Matsumura et al. (6) found that some LPS-treated pregnant rats showed the number of COX-2-positive cells well within the range observed in their nonpregnant counterparts but still exhibited a blunted PGE_2 response (Fig. 6). This finding suggests involvement of a COX-2-independent mechanism. The existence of such a mechanism is strongly evidenced by the attenuated thermal response of pregnant rats to central administration of PGE_2 (3, 11, 17).

The brain level of PGE_2 reflects not only synthesis but also clearance of this mediator from the brain through the choroid plexus with subsequent inactivation by the lungs and liver. Transport and inactivation of PGE_2 involve multiple proteins; the rate-limiting PGE_2-inactivating enzyme is 15-hydroxy-PG dehydrogenase (15-PGDH) (5). Noteworthy, pharmacological inhibition of PGE_2 efflux from the brain increases the pyrogenic activity of intrabrain PGE_2 (1). LPS-induced transcriptional downregulation of four PGE_2-transporting and -catabolizing proteins in the lungs and liver was found in our recent study (9); the gene suppressed most quickly (<30 min, latency) and most strongly (>25-fold) was 15-PGDH. Because the half-life of this enzyme is short, <50 min, transcriptional inhibition of 15-PGDH readily changes the protein level (2) and is likely to be of physiological significance for maintaining the febrile response (9).

Transport and catabolism of PGE_2 are affected by pregnancy, during which the uptake of $PGF_{2\alpha}$ by the choroid plexus is accelerated (10). A similar accelera-

tion of the brain-to-blood efflux should be expected for PGE_2, which is carried by the same transporters (15). Even more importantly, late pregnancy is accompanied by a strong transcriptional upregulation and dramatic (50-fold) increase in the activity of 15-PGDH in the lungs and other organs (13). That progesterone induces 15-PGDH expression (18) may provide a triggering mechanism for the upregulation of this enzyme.

We suggest that pregnancy-associated antipyresis reflects a facilitated efflux of PGE_2 from the brain with facilitated catabolism in the lungs and liver. Such facilitation is the result of the expressional upregulation of PGE_2 carriers and 15-PGDH. This hypothesis explains a wide range of phenomena observed in pregnant animals: the suppressed febrile response to peripheral LPS and cytokines (for review, see Refs. 6, 12), the blunted increase in brain PGE_2 in response to peripheral LPS (6) and cytokines (4), and the decreased thermal response to central PGE_2 (3, 11, 17). The facilitated transport and catabolism may play an adaptive role by protecting the body from the undesired systemic effects of PGs massively produced in the reproductive tract at near term.

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REPLY

To the Editor: Attenuation of fever at near-term pregnancy has been reported to occur in ewes (10), guinea pigs (21), and rats (12). Although more than 30 years have passed since the first report of this phenomenon by Kasting et al. (10), the mechanism underlying it is not yet fully understood. Recent papers from three independent groups (3, 6, 16) have shed light on this issue by showing an alteration in brain PGE_2 biosynthesis as a possible cause of suppressed fever in near-term rats. Imai-Matsumura et al. (6) and Fewell et al. (3) reported that near-term rats injected with either LPS or interleukin-1β showed lower PGE_2 levels in their brain extracellular fluid than nonpregnant female rats treated in the same way. Imai-Matsumura et al. (6) and Mouihate et al. (16) further showed LPS-induced cyclooxygenase-2 (COX-2) expression in the rat brain was blunted at near-term pregnancy. Inasmuch as COX-2 is one of the rate-limiting enzymes in PGE_2 biosynthesis and is essential to fever (1, 4, 11, 14, 15, 20), the above results suggest that blunted induction of brain COX-2 lowers the extracellular PGE_2 level, which, in turn, leads to suppression of fever in near-term rats.

In response to the above idea, Ivanov and Romanovsky raised the question as to whether the blunted COX-2 induction is really the cause of the lowered PGE_2 level. As the basis for this question, they cited two papers, one from their group (8) and one from our group (7), and stated, "recent in vivo data demon-

strate the lack of correlation between the tissue level of COX-2 and either the concentration of PGE_2 or the height of fever." We are afraid that this sentence is oversimplified and may mislead the readers. In fact, the study by Inoue et al. (7) showed a good correlation between COX-2 protein and PGE_2 level in a limited time window. In that study, LPS was injected into male adult rats intraperitoneally at a dose of 100 μg/kg. Their cerebrospinal fluid (CSF) and brain were sampled at seven time points, i.e., 0 min, 45 min, 1.5 h, 3 h, 5 h, 12 h, and 24 h after the LPS injection. Up to 3 h after the injection, the amount of induced COX-2 protein and CSF PGE_2 level correlated well. Thus the time point of 3 h taken by Mouihate et al. (16) was reasonable. In addition, Imai-Matsumura et al. (6) showed a good correlation between the PGE_2 level and the number of COX-2-positive cells at 4 h after LPS injection into female rats (Fig. 6 in the paper). In respect to this correlation plot, Ivanov and Romanovsky pointed out that one nonpregnant rat had a higher PGE_2 value with a smaller number of COX-2-positive cells than two of the pregnant rats, suggesting that the amount of COX-2 is not the major determinant of the PGE_2 level. However, because the number of animals analyzed was small, it is hard to draw any conclusion from one split point. Although it is possible that some factor other than COX-2 influenced the PGE_2 level around this time point, the correlation between COX-2 and PGE_2 was still good as a whole at 4 h after the LPS injection. Therefore, we consider that blunted COX-2 induction in the brain at near term is one of the major causes of the lowered PGE_2 level in the CSF.

On the other hand, Inoue et al. (7) showed that, at 5 h after the LPS injection, the PGE_2 level decreased by 50% from the level at 3 h, whereas COX-2 protein level was comparable to that at 3 h. Thus, if we expand the time window up to 5 h, "the lack of correlation" becomes apparent. We speculate that an additional mechanism that lowers the PGE_2 level was activated around 5 h after LPS injection and later. Perhaps it might be the so-called endogenous antipyretic mechanism, which may involve antipyretic peptides, glucocorticoid, PGE_2-catabolizing enzymes, P-450 products of arachidonic acid, or PGE_2 transporter for the clearance. This is another important issue for future study.

As an alternative hypothesis, Ivanov and Romanovsky proposed that accelerated PGE_2 catabolism at near term could be the cause of the lowered PGE_2 level in the brain and, thereby, the cause of suppressed fever. They recently demonstrated in male rats that intravenous injection of LPS downregulated PG-catabolizing enzymes and PG-transporting proteins in the lung and liver, but not in the brain (9). They speculated that reduced PGE_2 catabolism in the peripheral organs may cause an elevation in circulating PGE_2 level, which, in turn, reduces the brain-blood PGE_2 gradient. This may contribute to keeping high levels of PGE_2 in the brain during fever. In relation to near-term suppression of fever, they referred studies from other groups showing that PG dehydrogenase (PGDH), a major PGE_2-catabolizing enzyme, is upregulated in the lung and reproductive organs at near term in rabbits and rats (18). Upregulation of PGDH in these organs may lower the PGE_2 level in the circulation, increase the brain-blood PGE_2 gradient, and accelerate the clearance of PGE_2 from the brain. However, it should be noted that circulating levels of PGs, including PGE_2, increase dramatically during pregnancy (17, 19), probably because enhanced PG production in reproductive organs overwhelms PG catabolism by PGDH. If PGE_2 level increases in the arterial blood during pregnancy, the brain-blood PGE_2 gradient should be lower in pregnant animals than in nonpregnant ones. Unfortunately, as far as I know, there is no study that compared the arterial PGE_2 level between pyrogen-treated pregnant and non-pregnant animals. Thus the hypothesis by Ivanov and Romanovsky is intriguing but needs further verification. Pregnancy is accompanied by alterations in various physiological responses, including reduced febrile response to PGE_2 (2, 13) and suppressed thermogenesis in the cold (5). Therefore, it would be reasonable to consider that multiple mechanisms are involved in the near-term suppression of fever, and the suppressed COX-2 induction at near term is one of the major mechanisms. Whatever the truth may be, the argument by Ivanov and Romanovsky is of value because it reminds us that PGE_2 and fever should be discussed on the basis of the production, reception, and clearance of PGE_2.

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REPLY

To the Editor: The suppression of fever at near term appears to be a feature of most mammalian species studied to date (16), yet the mechanism responsible for this has eluded definition for over 20 years (19). Our recent demonstration (13) that both basal and LPS-stimulated cyclooxygenase-2 (COX-2) levels were reduced at near term provided a possible explanation for at least some of the reduced response. As COX-2 is considered the rate-limiting enzyme for the synthesis of PGE_2, we postulated that a reduction in the levels of COX-2, as demonstrated by semi-quantitative Western blot of hypothalamic proteins, would result in reduced synthesis of PGE_2 and consequently a reduced fever. This finding has now been corroborated in two other publications. Fewell and colleagues (5) carried out microdialysis of the preoptic area and analyzed PGE_2 levels in the dialysates in response to intravenous recombinant rat interleukin-1β. Whereas nonpregnant rats displayed increases in PGE_2 in concert with the elevation of body temperature [as reported previously by Komaki et al. (9)], rats at near term displayed neither a fever nor an elevation in PGE_2 levels. Similarly, a recent report by Imai-Matsumura et al. (6) reported reduced fever, reduced cerebrospinal fluid PGE_2 levels, and significantly fewer COX-2-immunoreactive endothelial cells in the preoptic area in response to LPS injection in rats at term. Thus three independent groups have almost simultaneously reported similar and complementary data and all have arrived at the same conclusion, namely that there is a suppression of COX-2 activity and concomitant PGE_2 synthesis at term. It is important to note that we and Imai-Matsumura and colleagues (6) both recognized that other factors, downstream from PGE_2 synthesis, could also be involved in the suppression of fever. For this reason, we also examined the levels of the PGE_2 receptor, EP_3 at term, but found that they did not change. In an editorial focus accompanying our publication, Roth and Persson (19) also suggest that there may be enhanced synthesis of endogenous antipyretics, another avenue we have also pursued (2).

Nonetheless, Ivanov and Romanovsky question both the correlation between COX-2 levels and the magnitude of fever and our conclusion that reduced PGE_2 levels, due to reduced COX-2 activity, are, in part, responsible for the reduced fever at term. They raise another possibility, that of accelerated catabolism or efflux of PGE_2. Their comments are welcomed, as we also feel that there may be more than one alteration in the cascade of events leading to fever that occur at term. However, some of their points reflect a possible misunderstanding of our data and the published literature and we will take this opportunity to clarify some of the issues they raised.

As they point out, the reduction in COX-2 levels we report is of the order of 40%, and they question whether this is sufficient to affect either the level of PGE_2 or the magnitude of fever. Although this is a valid consideration, it would appear that the data in the papers by Fewell et al. (5) and Imai-Matsumura et al. (6) clearly indicate that PGE_2 levels are indeed suppressed. Furthermore, Imai-Matsumura et al. (6) demonstrate an excellent correlation between PGE_2 levels in the cerebrospinal fluid and the numbers (and intensity of staining) of immunoreactive COX-2 cells after LPS. It is also noteworthy that COX-2 exists in the hypothalamus both in neurons under basal conditions (1) and in endothelial and perivascular cells where it is induced by inflammatory stimuli (10, 12, 18, 20). Our extraction of the entire basal hypothalamus and preoptic area undoubtedly included all of these cell populations, and the true reduction of COX-2 in the cells responsible for the PGE_2 production important in the febrile process is almost certainly much greater than that we were able to show.

Ivanov and Romanovsky also question a rate-limiting role of COX-2, as they cite references purporting to

demonstrate a lack of correlation between the concentration of brain PGE_2 and the magnitude of the fever. This appears to be a misinterpretation of the data in these and other papers dealing with this issue. Matsumura et al. (12) reported an excellent correlation between COX-2 levels and the height and duration of fever (see their Fig. 7), a finding complimented by a report that COX-2 inhibitors simultaneously suppressed both cerebrospinal PGE_2 levels and fever (22). Even the papers (7, 8) cited by Ivanov and Romanovsky both report an excellent temporal relation between the expression of COX-2 in endothelial cells, the elevation of PGE_2 in cerebrospinal fluid and the onset of fever at the time points when we collected our tissue (3 h after LPS). Where the relationship between these factors is altered appears after the fever is entering a defervescence stage, when endogenous antipyretics may become involved (3).

The alternate mechanism proposed by Ivanov and Romanovsky, that of increased catabolism and transport of PGE_2 from the brain, could indeed contribute to the reduced fevers, given that prostaglandins involved in fever appear to be inactivated via an efflux from the hypothalamus (4, 21). Furthermore, such a mechanism would be compatible with our observations of reduced central response to PGE_2 at near term (2, 11). However, the role in fever suppression of catabolism of PGE_2 by the major catabolizing enzyme 15-hydroxy-PG dehydrogenase (15-PGDH) is still an open question. This enzyme is induced by progesterone, a hormone whose levels and activity vary dramatically during pregnancy. If this enzyme is indeed important in controlling PGE_2 levels (and by extension, fever magnitude), it is curious that at gestational *day 15*, when there is little fever suppression, progesterone levels are at their highest in the rat, whereas at term, progesterone levels have declined precipitously (15). In line with this observation, and in contrast to what is suggested by Ivanov and Romanovsky, it has been reported that 15-PDGH activity in a number of rat tissues decreases significantly at parturition (14). Thus these facts cast some doubt on an obligatory role for enhanced 15-PDGH activity in the suppression of fever at term. However, as much of the data on the role(s) and regulation of this and other catabolic enzymes during pregnancy have been obtained in other species, in nonneuronal tissue, or from tissue in vitro, this is an area requiring further study.

Also in need of further study is an examination of the expression, throughout pregnancy, of other EP receptors and central nervous system transmitters thought to be involved in thermogenesis (17). It will also be important to determine the mechanism for the suppressed COX-2 induction, which may be due to alterations in levels of both inflammatory and anti-inflammatory cytokines or hormonal action on upstream regulators of COX-2. Our demonstration of suppressed COX-2 represents but the first step in our understanding of this fascinating response.

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