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Prostaglandin E_2 And Fever: A Continuing Debate

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Prostaglandin (PG) E_2 is a potent hyperthermic agent and has been assigned an intermediary function in the response of thermoregulatory neurons to pyrogens. Though attractive, this idea has been challenged on several grounds. The present study confirms that brain PGE_2 synthesis increases during fever, the time course of the elevation according with a causative role of the compound. Our experimental data also argue against the involvement of a second cyclooxygenase product, specifically thromboxane (TX) A_2, in the action of pyrogens. The sequence of events leading to PGE_2 production and fever differs depending on the pyrogen, bacterial vs. leucocytic, and its route of administration. Blood-borne interleukin-1 (IL-1) acts on a discrete site in the central nervous system (CNS) which is tentatively identified with the *organum vasculosum laminae terminalis* (OVLT). The same site may also be the target for blood-borne endotoxin. In addition, endotoxin may promote PGE_2 synthesis in the cerebral microvasculature. Both pyrogens, on the other hand, act diffusely throughout the CNS when given intrathecally. We conclude that PGE_2 is well suited for an intermediary role in the genesis of fever and ascribe the reported inconsistencies to methodological factors.

Considerable evidence implicates a product of arachidonate cyclooxygenase, specifically prostaglandin (PG) E_2, in the genesis of pyrogen fever [1]. According to this proposal, PGE_2 formed within the brain in response to blood-borne interleukin-1 (IL-1), and possibly endotoxin as well, acts at an appropriate place in the thermoregulatory pathways to move upward the "set-point" around which temperature is regulated. Though attractive, this scheme has been challenged by several investigators and their arguments are summarized in Table 1. Briefly, inconsistent findings fall under three broad categories dealing with, respectively, the lack of correlation between the occurrence of fever and rate of PGE_2 synthesis (Table 1, *a* to *d*), the involvement of a second cyclooxygenase product acting in concert with, or substitution of, PGE_2 as a mediator of fever (Table 1, *e* to *g*), and the uncertainty on the location of the site, or sites, in which pyrogen action is translated into enhanced PGE_2 formation (Table 1, *h*).

We will focus this paper on a few issues, currently addressed in our laboratory, that need to be settled before accepting the scheme under debate. They include the question of the variability in the reported levels of PGE_2 in the cerebrospinal fluid (CSF), the feasibility of thromboxane (TX) A_2 acting as a fever mediator, and the ability of blood-borne pyrogens to promote PGE_2 synthesis at the blood-brain interface and in the substance of the brain. Other issues are adequately covered in the remaining papers of this section.

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TABLE 1
Evidence Against a Role of PGE_2 in the Mediation of Pyrogen Fever

Finding	Reference
a. Reported PGE_2 levels in CSF are variable and the range of values under afebrile and febrile conditions overlap	[2–8]
b. Rate of PGE_2 release in the rostral hypothalamus does not correlate with the fever following intravenous endotoxin	[9]
c. Salicylate at an appropriate dose prevents PGE_2 elevation in the CSF but not the fever response to intravenous IL-1	[5]
d. Inhibition of protein synthesis attenuates fever to systemic pyrogens without causing a concomitant fall in PGE_2 levels in the CSF	[4]
e. Prostaglandin antagonists interfere with the fever to intraventricular PGE_2 while leaving pyrogen action unaffected	[10]
f. Animals with extensive lesions of the rostral hypothalamus develop fever to intravenous pyrogen but not to intrathecal PGE_2	[9]
g. Newborns may respond to pyrogens but not PGE_2	[11]
h. Systemic pyrogens, bacterial and leucocytic, promote PGE_2 synthesis in brain despite their apparent inability to cross the blood-brain barrier	[12–14]

PGE_2 IN CEREBROSPINAL FLUID: AFEBRILE VS. FEBRILE STATE

A precise measurement of PGE_2 levels in CSF is crucial for the understanding of the sequence of events leading to fever. Unhappily, many published figures have been obtained prior to the refinement of analytical methods, using a biological assay or immunological assays in which either the antibody was unspecific or chemical transformation of PGE_2 to PGB_2 was required. Not surprisingly, PGE_2 values are widely scattered and, though pointing to an elevation during fever, are not entirely convincing.

We have re-examined this issue using conscious cats with a sampling cannula chronically implanted inside the third ventricle and have shown that PGE_2 is mostly undetectable (<100 pg/ml) when CSF is collected in the absence of fever. Our finding is at variance with earlier data and has been discussed elsewhere [15]. At the same time, we have found that PGE_2 content of the CSF is variably altered by fever, depending on the pyrogen used and its route of administration.

Bolus injection of IL-1 into the third ventricle caused fever and stimulation of PGE_2 synthesis. The latter effect was reflected in fewer samples with subthreshold activity and sustained rise in the measured levels of the compound (about 800 pg/ml). PGE_2 elevation was instead marginal during the monophasic fever following intravenous bolus injection of IL-1.

Unlike IL-1, endotoxin could clearly promote PGE_2 synthesis by either route, though magnitude and consistency of effects were greater with the intrathecal route. When given intravenously, endotoxin was effective in about 60 percent of the experiments, and its action persisted throughout the fever. The reason for the failures is not clear; however, the position of the sampling cannula may be important since highest PGE_2 values (maximum, 1.1 ng/ml) were obtained with the cannula located closer to the anterior recess of the third ventricle. Intracerebroventricular endotoxin, on the other hand, mimicked IL-1 in causing a sustained PGE_2 elevation in all cases. Significantly, this elevation preceded the onset of the fever.

The discrete change in PGE_2 following an intravenous bolus of IL-1 cannot be

ascribed to the short half-life of the pyrogen in the circulation, and the attendant transient action on the central nervous system (CNS), because similar results were obtained in experiments in which IL-1 was administered by continuous intravenous infusion and the resulting fever was sustained. On the other hand, brain tissue has little, if any, prostaglandin catabolic activity [16] and no significant breakdown of PGE_2 is expected under any condition. Indeed, separate experiments proved that 15-keto-13, 14-dihydro PGE_2 is not detectable (<140 pg/ml) in CSF collected both in the absence and the presence of fever.

We conclude that endotoxin is a better stimulus than IL-1 in eliciting PGE_2 synthesis by the intravenous route, suggesting a more direct, or diffuse, action for that pyrogen on the CNS. Both pyrogens promote PGE_2 synthesis by the intrathecal route and, consistent with a mediator role for this prostaglandin, the effect is already detected during the latent period of the fever. Overall, our values for PGE_2 content of the CSF are lower than those reported in early studies, and this finding introduces an element of doubt in certain data (Table 1, *c* and *d*) purportedly contradicting the PGE_2 mediator idea. In fact, contrary to the evidence presented in Table 1, recent work employing a specific radioimmunoassay procedure has shown that pyrogen fever and PGE_2 elevation abate in parallel after administration of a protein synthesis inhibitor [17].

THROMBOXANE A_2 AND FEVER

Under certain conditions, pyrogens remain effective despite the apparent lack of a functional PGE_2 mechanism (Table 1, *e* to *g*) and the resulting fever is susceptible to antipyretic treatment. This observation would imply that a second cyclooxygenase product acts as a fever mediator and, mainly by exclusion, TXA_2 has been considered a suitable candidate. In support of this view are the occurrence of active TXA_2 synthesis in the brain parenchyma [16] and the notion that stable endoperoxide analogs, which are known to behave as TXA_2 mimics in other systems, may alter body temperature [18,19].

We have examined this possibility and initial results, particularly the demonstration of an antipyretic action for imidazole, were promising [15]. However, our recent work does not provide support for this idea and specific findings are summarized below.

1. A stable TXA_2 analog, 9,11-epithio-11,12-methano-TXA_2 (ONO-11113, 2 μg IVT), produced variable changes in body temperature (hyper- or hypothermia) or no effect at all.

2. Fever to intracerebroventricular endotoxin was not modified by treatment with the thromboxane antagonist, 9,11-dimethylmethano-11,12-methano-16-phenyl-13,14-dihydro-13-aza-15$\alpha\beta$-ω-tetranor-TXA_2 (ONO-11120). The compound (2 μg IVT) was given five or 35 minutes after endotoxin with identical results.

In addition, we found that intravenous pyrogen, both bacterial and leucocytic, is consistently without effect on TXB_2[1] levels in the CSF. Intracerebroventricular pyrogen, on the other hand, promoted thromboxane synthesis, but stimulation was limited to an early stage of the fever response. Both results differ from those obtained with PGE_2.

While arguing against the involvement of TXA_2, the above findings leave open the question of an alternative fever mediator being formed in the cyclooxygenase pathway.

[1]TXB_2 is the stable byproduct of TXA_2 and its measurement provides an index of the rate of thromboxane synthesis.

At the same time, however, they call for a closer scrutiny of the evidence supporting the existence of such mediator. For example, PGE_2 antagonists are poorly soluble in aqueous media and, when injected intraventricularly, may affect differently responses to the exogenous and endogenous prostaglandin. Likewise, the apparent lack of effect of intraventricular PGE_2 in animals with extensive lesions of the rostral hypothalamus could simply reflect impaired diffusion of the compound to the target site. Unfortunately, no information is available on the ability of these animals to develop fever in response to systemic PGE_2. Lastly, analysis of results in the newborn must take into account the existence of an endogenous antipyretic principle, possibly identified with arginine vasopressin [20]. Such an agent may interact in a variable manner with pyrogens and PGE_2 during the early neonatal period. All these questions need to be answered before again approaching the problem of a second fever mediator.

SITE OF PYROGEN ACTION IN THE CNS

Blood-borne IL-1 is conventionally regarded as the common intermediary for a multiplicity of fever-producing agents and conditions. Nevertheless, its mode of action on the CNS is unclear because, on the one hand, the blood-brain barrier is seemingly impermeable to pyrogens [12–14] and, on the other hand, intrathecal administration of a cyclooxygenase inhibitor results in suppression of fever [21]. The latter finding implies that pyrogen action is exerted within the brain parenchyma or, perhaps, in the microvasculature. Alternatively, the pyrogen could act outside the blood-brain barrier in a circumventricular organ. One such organ, the *organum vasculosum laminae terminalis* (OVLT), is connected with the median pre-optic area and is ascribed a role in the genesis of fever [22]. In fact, our data suggest that IL-1 action is confined to a discrete brain region.

To gather more information on the location of IL-1-responsive site(s), we have studied the effect of intravenously infused IL-1 in cats pretreated with probenecid (30 mg/kg IP or IV; 50 or 100 μg IVT). Probenecid interferes with the transport of prostaglandins from brain to blood [23] and, therefore, would seem a suitable tool for "magnifying" any PGE_2-linked effect of IL-1. However, findings did not differ from those obtained in naive animals, and PGE_2 elevation remained marginal throughout the fever.

A more direct approach to this problem was taken in subsequent experiments in which PGE_2 release was monitored *in vitro* and *in vivo* using, respectively, isolated cerebral microvessels and "push-pull" perfusion cannulas positioned inside the rostral hypothalamus.

Cerebral Microvessels

The idea of the intraparenchymal microvasculature being the target for blood-borne pyrogens is conceptually appealing and also accords with some experimental observations. The hypothalamus is richly vascularized and blood vessels are endowed with an active prostaglandin-generating system [24]. Furthermore, hypothalamic blood flow is increased during fever [25], possibly reflecting stimulation of prostaglandin synthesis in the vessel wall.

Cerebral microvessels, consisting mostly of capillaries, released PGE_2 in the amount of about 70 pg/mg protein/minute and this release rate doubled during exposure to endotoxin (10 μg/ml). IL-1 (maximum, one rabbit pyrogenic dose/ml) had instead an opposite effect. While the inhibitory action of IL-1 remains unexplained, the stimula-

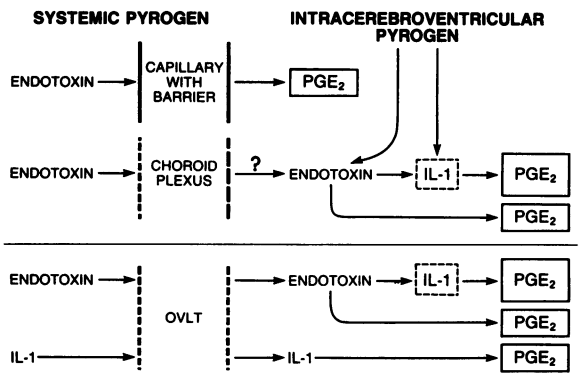

FIG. 1. Hypothesis on the mechanism by which pyrogens, bacterial and leucocytic (IL-1), promote PGE_2 synthesis in the central nervous system, thus causing fever. A different sequence of events is envisaged depending on whether the pyrogen is given systemically or intrathecally. Note that brain may produce IL-1 (*shown within interrupted lines*) upon direct endotoxin challenge. **OVLT**, *organum vasculosum laminae terminalis.*

tory action of endotoxin, if representative of the condition *in vivo*, could account for the observed rise in PGE_2 content of the CSF.

Local Perfusion of the Hypothalamus

PGE_2 release was monitored at discrete hypothalamic sites, using a modified "push-pull" perfusion procedure in which stringent precautions were taken to avoid damage to the tissue. In the absence of fever, the release rate was generally below detection (<0.5 pg/minute), but rose to measurable levels in most experiments in which probenecid was added to the perfusion fluid (final concentration, 1 mM). Even during local probenecid treatment, however, intravenous IL-1 caused only a slight increase in the concentration of PGE_2 in the effluent. Unfortunately, sites studied so far were located mostly in the anterior hypothalamus and inadequate screening of the pre-optic area could account for this modest response.

CONCLUSION

The arguments listed in Table 1 form collectively a convincing case against the existence of a "PGE_2 link" in the central action of pyrogens. Their significance, however, is greatly weakened by methodological problems and incomplete experimental verification. Equally debatable is the occurrence of a second cyclooxygenase product with pyrogenic properties. In our view, PGE_2 remains the best candidate for mediating the response of thermoregulatory neurons to pyrogens, and a tentative scheme integrating our data as well as data of others is given in Fig.1. Systemic endotoxin may promote PGE_2 synthesis in brain capillaries. It may also be possible for endotoxin, or rather an active fragment derived from its breakdown, to cross the choroidal barrier in small amounts. The action of this pyrogenic moiety would be "amplified" within the confines of brain since our current work indicates the appearance of IL-1 in CSF of cats treated with intracerebroventricular endotoxin [Coceani F, Dinarello C, Lees J: unpublished]. Both endotoxin and IL-1 gain access to neurons in the OVLT and may initiate a sequence of events leading to the local formation of PGE_2 in the rostral hypothalamus. Intraventricularly injected pyrogens, on the other hand, act diffusely throughout the brain.

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