ROLE OF PROSTAGLANDIN E IN THE BIPHASIC
FEVER RESPONSE TO ENDOTOXIN*

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Intravascular injections or infusions of moderate doses of bacterial pyrogens are known to produce biphasic fevers, whereas minimal doses elicit monophasic fevers (1, 2). Pyrogen injections into various areas of the brain generally result in monophasic fevers, although recently, biphasic fever has been observed after injections of bacterial pyrogens into the anterior hypothalamic, preoptic region (3). Although it is known that the second phase of fever is associated temporally with circulating leukocyte pyrogen, host mechanisms responsible for the two phases of the biphasic response are not understood (1, 4).

A large body of evidence has accumulated recently that supports the view that prostaglandin E (PGE)[1] is a primary mediator of fevers induced by bacterial pyrogens (5–8). Fevers evoked by injections of enteric bacteria either into the third ventricle or proximate to the anterior hypothalamus of the cat (9) are associated with increased PGE levels in cerebrospinal fluid (CSF). Fever, as well as PGE concentrations in CSF, decrease after treatment of febrile animals with antipyretic compounds (3, 10) known to inhibit prostaglandin synthesis (11). Additional supportive evidence (6, 12) shows that direct injections of E prostaglandins into the cerebral ventricles of cats and rabbits elicit abrupt, monophasic fevers.

More recently, however, the view that PGE is the principal mediator of fever induced by leukocyte pyrogen (LP) has been challenged by Cranston et al. (13, 14), who effectively dissociated PGE levels in CSF from the fever response to intravenous infusions of LP. They also found that, although intraventricular injections of PG antagonists together with PGE attenuated fever, little or no change in fever response resulted when these antagonists were injected simultaneously with LP. Other recent evidence (15) casts doubt on the role of PGE in both endotoxin and LP-induced fevers because pretreatment of rabbits with cycloheximide prevented fever in rabbits, even though PGE concentrations in CSF increased to the levels attained in febrile animals not treated with the protein synthesis inhibitor. However, a clear interpretation of the latter experiment is complicated by the fact that cycloheximide alone caused a significant decrement in the core temperature of test animals.

We investigated fever responses in the sheep after the intravascular administration of endotoxin and LP with the objective of determining whether PGE or certain other oxidation products of arachidonic acid participate in the fever reaction. Part of this work has been presented elsewhere (16).

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[1] *Abbreviations used in this paper:* CSF, cerebrospinal fluid; PG, prostaglandin; LP, leukocyte pyrogen; RIA, radioimmunoassay.

Materials and Methods

Experimental Model. A group of six anestrous Merino ewes, weighing between 40 and 60 kg, were used as subjects; all had equal amounts of wool, having been shorn on the same day several months earlier. At least 6 mo before the start of the experiments, a carotid artery and jugular vein were exteriorized and enclosed in a loop of skin from the neck, according to the procedure of McDonald et al. (17). Before experimental work, animals were conditioned in steel metabolism cages in a temperature-regulated room (23 ± 1.5°C). At least one other animal was present in the room to prevent isolation stress to which flock animals are susceptible.

To minimize the stress of blood sampling, infusions, and injections, sterile probes and cannulae were positioned on the evening before each experiment. Catheters (Becton, Dickinson & Co., Rutherford, N. J.; 20 gauge, 2 in) were placed in the carotid artery and jugular vein (within the exteriorized skin loop) and sutured in place using aseptic technique. A Deseret E-Z catheter (Deseret Pharmaceutical Co., Sandy, Utah; 16 gauge, 8 in) was inserted in the *in situ* jugular vein and sutured in place. A Teflon cannula (16 gauge, 4 in) was placed subcutaneously in the flank area for the administration of indomethacin. To prevent clotting, the animals were given 20,000 IU of Depot-Heparin (Upjohn Co., Kalamazoo, Mich.). All cannulae were filled with heparin (1,000 IU/ml) and closed with three-way stopcocks.

Blood pressure was monitored in the exteriorized carotid artery using a conventional transducer and preamplifier (Biotronex Laboratory, Inc., Kensington, Md.; ΔBL-630). Flexible Teflon tubing (OD, 3.25 mm; ID, 2.0 mm) ~1 m in length connected the intra-arterial cannula to the transducer. The output of the preamplifier was fed into a Grass polygraph (Grass Instrument Co., Quincy, Mass.) where mean arterial blood pressure was recorded. A flexible Tele thermometer thermistor (Yellow Springs Instrument Co., Yellow Springs, Ohio) was inserted 7–10 cm into the vagina and secured in position by sutures anchored in the adjacent wool. The output from a thermogauge (Yellow Springs Instrument Co.; model 43 TA) was fed into the polygraph and used to monitor the core temperature. Temperature, mean arterial blood pressure, and time were recorded simultaneously. The blood pressure transducer was calibrated by means of a sphygmomanometer connected at a T junction on the transducer. To insure accuracy of the results, thermogauge and blood pressure calibrations were made before and after each experiment.

Approximately 1 h before each experiment, 8,000 IU of heparin was given intravenously, followed by 5,000 IU every 3 h thereafter. When required, indomethacin was dissolved in sesame oil (10 mg/ml) and administered subcutaneously 1 h before injection or infusion of the pyrogen.

Infusions. Generally, 1-h infusions of 20–30 ml of normal saline containing endotoxin, LP, or PG were administered with a Harvard infusion pump (Harvard Apparatus Co., Inc., S. Natick, Mass.). A Millipore membrane (0.45 μm; Millipore Corp., Bedford, Mass.) was fixed to the hub of the infusion syringe, and sterile Teflon tubing was used to connect the syringe to the intra-arterial catheter. Control infusions of sterile saline were given routinely to establish baseline temperature and blood pressure; both were monitored continuously before, during, and after the various infusions. For some experiments, hind-limb infusions of endotoxin were administered through a cannula in the left femoral artery. In such instances, the left femoral vein was also cannulated to permit blood collections from this site as well as from the exteriorized carotid artery and jugular vein.

Blood Collections. Blood samples (3.5 ml) were withdrawn simultaneously from the indwelling catheters in the exteriorized carotid artery and the jugular vein. All blood samples were put immediately into iced tubes containing 10 μg of indomethacin. Shortly after collection, blood samples were centrifuged for 10 min at 1,800 rpm, and the plasma supernates were centrifuged a second time to remove any remaining cells. The clarified plasmas were stored in closed tubes at −15°C and were later analyzed for PGE and PGF content by radioimmunoassay (RIA). At the end of these experiments, animals were given 250 ml of normal whole blood and were rested for at least 3 wk before reuse.

Endotoxin. A Boivin preparation of endotoxin (*Salmonella enteritidis*; Difco Laboratories, Detroit, Mich.) was dissolved in normal saline at 1 mg/ml and stored at −15°C. Before use, the stock solution was diluted in isotonic saline.

Prostaglandins. PGE_2, $PGF_{2\alpha}$, and PGD_2 were donated by Dr. John Pike (Upjohn Co.,

Kalamazoo, Mich.), and prostacyclin (PGI$_2$) was a gift from Dr. Joseph Fried, University of Chicago, Ill. Indomethacin was donated by the Merck Chemical Div., Merck & Co., Inc., Rahway, N. J., and ^{3}H-PGE$_1$ (60 Ci/mM) and ^{3}H-PGF$_{2\alpha}$ (10.7 Ci/mM) were purchased from New England Nuclear, Boston, Mass. With the exception of prostacyclin (PGI$_2$), the prostaglandins were dissolved in 95% ethanol at 1 mg/ml and stored at $-15°C$. On the ₊day of the experiment, the required amount of PG was removed and suitably diluted in pyrogen-free isotonic saline. A stock solution (1 mg/ml) of PGI$_2$ was made up in 15 mM Tris-phosphate buffer at pH 9.5 and used within 30 min of preparation. Immediately before infusion, the PGI$_2$ solution was suitably diluted in isotonic saline adjusted to pH 9.5 with 0.1 N NaOH.

LP. Peritoneal exudates were induced in the sheep by the intraperitoneal injection of 3 liters of normal saline containing 8 μg of *S. enteritidis* endotoxin. 12-14 h later, such animals received an infusion of 2.5 liters i. p. of warmed saline containing 10,000 IU of heparin. Cellular exudates were then drained from the peritoneal cavity through a 13-gauge needle. After light centrifugation, cell pellets were washed once with phosphate-buffered saline (10 mM, pH 7.4) and resuspended in 200 ml fresh buffered saline. The cells were incubated for 2 h at 37°C before rupture by five freeze-thaw cycles. Cellular debris was removed by centrifugation for 30 min at 5,000 rpm; the clear supernate represented the crude extract of LP. 25 ml of LP extract from $\sim2 \times 10^9$ exudate cells (90-94% granulocytes) produced a fever of 0.9-1.2°C.

RIA. This procedure has been described elsewhere (18, 19), although the following modifications were made for this study. Reproducibility of the RIA was verified on both unextracted and ethyl acetate-extracted plasma samples containing known quantities of PGE$_2$ or PGF$_{2\alpha}$. In contrast to our experience with serum or plasma from several other mammalian species, results were more consistent using unextracted sheep plasma, and the RIA data for this study were so derived. Before assaying, all test plasmas were spiked with 100 pg of the PG being measured to insure that measurements of test samples were obtained from the linear part of standard curves. Corrected PG values are expressed as the average of duplicate assays on 0.4-ml vol of each test plasma. Standard curves were run each day of assay in the presence of 0.4-ml aliquots of a stock supply of normal sheep plasma containing no measurable PG. The tracers used for estimation of PGE and PGF equivalents were ^{3}H-PGE$_1$ and ^{3}H-PGF$_{2\alpha}$, respectively.

Results

Fever Response to Endotoxin. To establish the appropriate pyrogenic dose in the sheep model, the endotoxin was given intravascularly, i.e., by the carotid artery or jugular vein, either as a slow infusion or as a single, brief injection. Doses of 1-2 μg per animal produced monophasic fevers that averaged 1.2°C, with a 42-min delay to onset of fever ($n = 9$). Higher doses, 4-10 μg, evoked biphasic fevers averaging 2.1°C, with a 26-min latent period ($n = 10$). The route and manner of endotoxin administration made little difference in the fever response except that infusions resulted in a slightly longer latent period.

Representative biphasic fever responses to intracarotid and jugular vein administration of endotoxin are recorded in Figs. 1 and 2. As is the case with other species, the onset of fever was accompanied by shivering. More notable, however, was the abrupt increase in systemic arterial blood pressure that invariably accompanied fever onset. This pressor response ranged from 10 to 20% as the dose of endotoxin increased from 1 to 10 μg. Blood pressure, which rose sharply during the first 10 min of fever, gradually diminished during the initial phase and remained normal throughout the second phase of fever.

PG have been reputed to mediate fever and inasmuch as we (20) and others (21-23) have shown that PG appear in the circulation after endotoxin challenge, blood samples were taken simultaneously from the carotid artery and jugular vein at frequent intervals before and during fevers induced by injections or infusions of 4-10 μg of endotoxin. In 12 separate experiments, RIA of plasma samples from the carotid
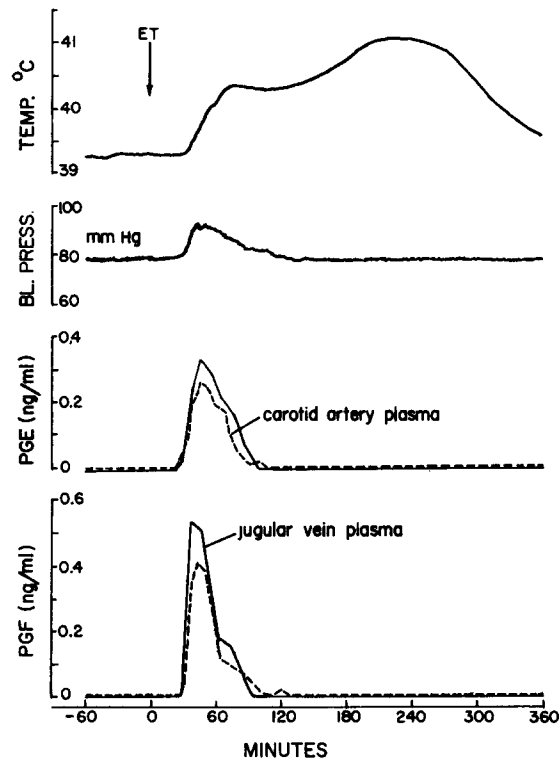
FIG. 1.   Fever and pressor responses to an intracarotid injection of 6 μg of endotoxin (ET). The plasma levels of PGE and PGF were determined by RIA. Blood samples were withdrawn simultaneously from the carotid artery and jugular vein at 5-min intervals (0–120 min) or 10-min intervals (−60 to −5 min and 130–400 min).

artery and jugular vein affirmed the appearance of PGF and PGE coincident with the onset of fever and pressor responses. From the data in Figs. 1 and 2 it is clear that (a) the surge of PG correlated well with the initial fever peak (~90 min), and (b) PG were not detected throughout the second phase of fever. In two of these experiments, additional blood samples were taken from both the femoral artery and femoral vein. RIA on plasma from this distal site also revealed the same surge of PGE and PGF coincident with the first peak of fever (Fig. 3).

The transitory appearance of PG in both arterial and venous plasma suggested that a generalized intravascular synthesis and release of these compounds occurred in response to endotoxin. To examine this possibility further, hind-limb infusions of small doses of endotoxin (1–1.5 μg) were given via the femoral artery. Blood plasma samples obtained simultaneously from the femoral vein, carotid artery, and jugular vein were subjected to RIA. Representative results of these assays are shown in Fig. 4, where it is seen that PGE appeared in plasma from the femoral and jugular veins ~30 min from the start of infusion. The time of appearance of PG in the venous circulation coincided with the rise in temperature and blood pressure. However, these small doses of endotoxin elicited barely detectable amounts of PG in carotid arterial plasma.

Fever Response to PG.   The appearance of PGE and PGF in the general circulation
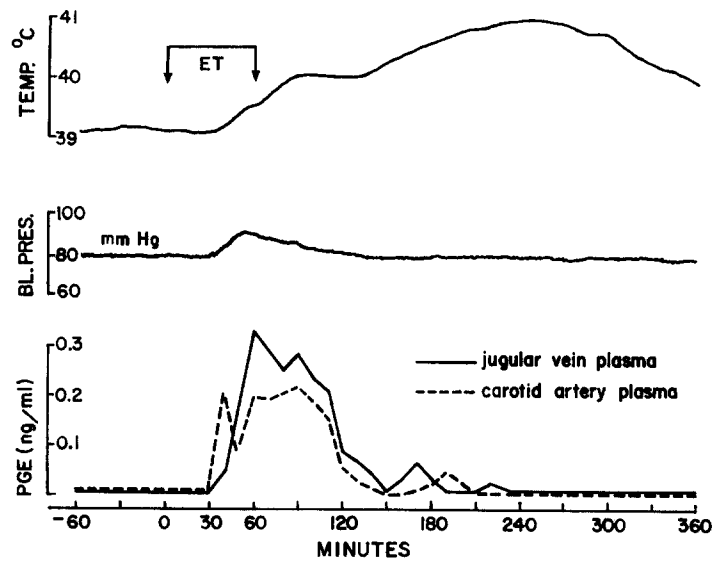
FIG. 2. Fever and pressor responses to a jugular vein infusion of 6 μg of endotoxin (ET). Blood samples for PG assays were collected from the carotid artery and jugular vein at 10-min intervals throughout the experiment. A surge of PGF in arterial and venous plasma during the initial fever (not shown) closely paralleled that of PGE.
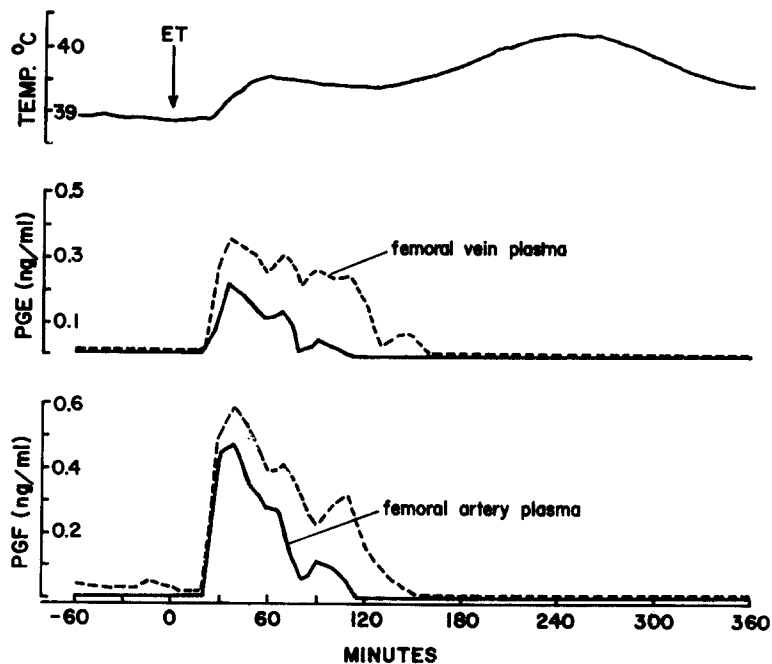
FIG. 3. Fever response to an intracarotid injection of 10 μg of endotoxin (ET). Blood collections for the PG assay were taken simultaneously from the femoral artery and femoral vein at 5-min intervals (0-120 min) or 10-min intervals(−60 to −5 min and 130-360 min).
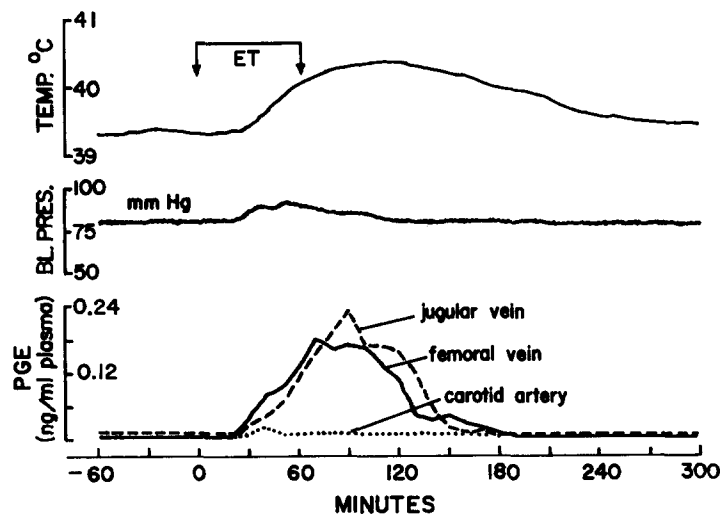
Fig. 4. Fever and pressor responses to a femoral artery infusion of 1.5 μg of endotoxin (ET). Simultaneous blood collections for PGE assay were taken at 10-min intervals from the jugular vein, femoral vein, and carotid artery.

during the early phase of endotoxin fever led us to investigate the pyrogenic effects of infusions of exogenous PG. Intravenous infusions did not alter core temperature or blood pressure, presumably because of the rapid metabolism of PG by the liver and lung (24, 25). Intracarotid infusions of PGE_2, however, evoked abrupt fever and blood pressure responses at doses ranging from 60 to 160 μg (1-2.7 μg/min). By contrast, carotid arterial infusions of 60–1,500 μg (1–25 μg/min) of $PGF_{2\alpha}$ consistently produced nominal decrements in core temperature (0.1–0.2°C) and had no effect on blood pressure (Fig. 5).

The fever response to PGE_2 was monophasic, with an average duration of ~3 h. Infusions of 80–100 μg (1.3–1.7 μg/min) produced fevers averaging 0.9°C, with a time of onset of 5–8 min (n = 7); 140–160 μg (2.3–2.7 μg/min) evoked fevers of 1.2°C, with a time of onset of 3–5 min (n = 7). The pressor response to PGE_2 was slightly faster, occurring within 1–3 min of the start of infusion. The magnitude of the blood pressure increment ranged from 10 to 25% in a dose-dependent manner.

Indomethacin Inhibition of Fever. The preceding results clearly imply an essential role for PGE in the initial fever and pressor responses to circulating endotoxin. Further support for this interpretation was obtained in experiments with indomethacin, a potent inhibitor of arachidonate metabolism. Because intra-arterial infusions of 5–10 μg/ml of indomethacin together with endotoxin had little or no effect on the fever response, indomethacin was given subcutaneously in sesame oil before infusions of the toxin. Complete inhibition of both fever and pressor responses was obtained consistently with a single dose of 4 mg/kg of indomethacin given 1 h before endotoxin challenge (Fig. 6). Lesser amounts of indomethacin (2–3 mg/kg) resulted in partial attenuation of fever, whereas no effect was seen at 1 mg/kg. PG were not measureable in carotid artery and jugular vein plasma from endotoxin-treated animals receiving 4 mg/kg indomethacin, nor was a pressor effect observed. Control subjects pretreated
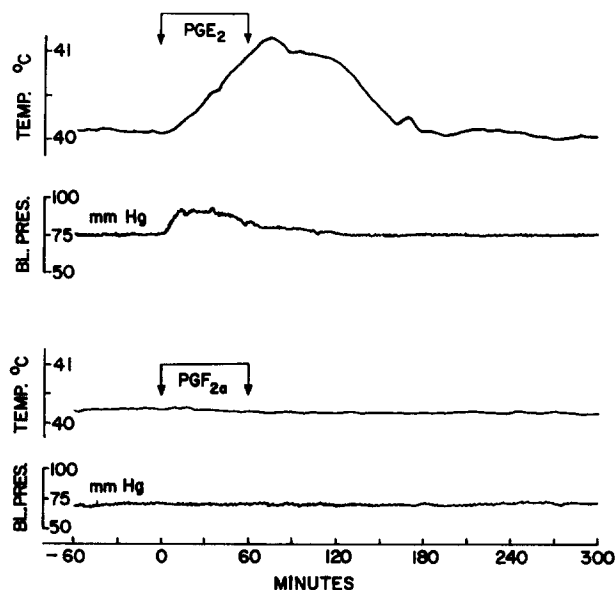
Fig. 5. Body temperature and pressor responses to intracarotid infusions of PGE$_2$ (2.6 µg/min) and PGF$_{2\alpha}$ (1.3–25.0 µg/min).
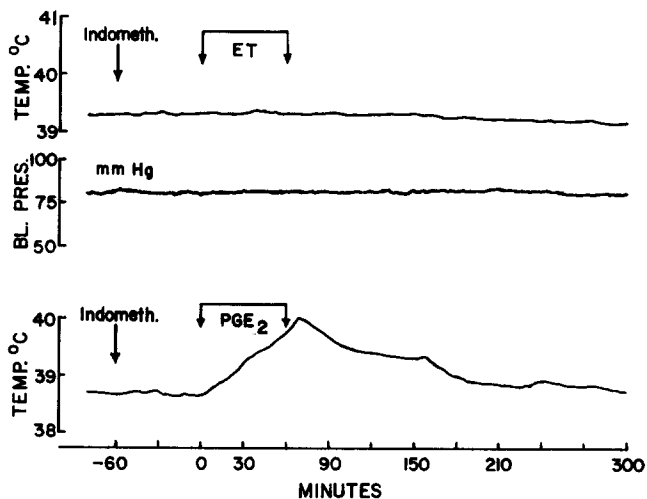

Fig. 6. Effect of indomethacin pretreatment (4 mg/kg, subcutaneously) on the fever and pressor responses to an intracarotid infusion of 10 µg of endotoxin (ET). PG assays of carotid artery and jugular vein plasma collected at 10-min intervals throughout the experiment indicated the absence of PGE and PGF. In control animals pretreated with indomethacin, both fever and pressor responses to intracarotid infusions of PGE$_2$ remained unchanged.

with this amount of indomethacin responded in characteristic fashion to infusions of PGE$_2$, as indicated in Fig. 6.

Fever Response to LP. Our results show that neither PGE nor PGF mediate the second phase of the biphasic fever response to endotoxin. However, the finding that indomethacin blocked the total fever response suggested a role for some other pyrogenic metabolite of arachidonic acid in the late phase. To clarify this point, we

investigated the fever response of the sheep to LP, the circulating endogenous protein(s) associated with the second phase of endotoxin-induced fever.

Intracarotid infusions of crude LP gave rise to monophasic fevers averaging 1.0°C, with latencies of 15–20 min (n = 6). Blood pressure changes were not seen, and frequent blood collections from the carotid artery and jugular vein before and during fever episodes showed the absence of PGE and PGF in plasma samples subjected to RIA. Pretreatment of animals with indomethacin abolished the fever response, as seen in Fig. 7.

The inhibition by indomethacin of both endotoxin and LP fever responses prompted us to investigate the possible pyrogenic action of other available metabolites of arachidonic acid. Carotid arterial infusions of prostacyclin (PGI₂) produced neither fever nor pressor effects at doses of 2.5 or 6.6 μg/min, although cardiac arrhythmia was observed at the higher concentration. Infusions of 2.5 or 6.6 μg/min of PGD₂ did not produce fever but did evoke pressor responses equivalent to those of PGE₂. Representative results from these experiments are given in Fig. 8.

Discussion

The role of PG in the pathogenesis of fever induced by endotoxins or leukocyte (endogenous) pyrogens is currently unresolved. Recent work on this subject has led to discordant observations and interpretations, which may relate to the common practice of administering pyrogens by extravascular route. In the present study, pyrogenic agents were given intravascularly by slow infusions or brief injections to simulate events that might occur during the course of an enteric bacteremia. This approach proved fruitful in that a clear delineation of the two phases of the biphasic fever response was made possible.

The use of the sheep as a model for this study was particularly advantageous for the following reasons. The fever response was monitored in conscious, conditioned animals, and injections, infusions, and blood sampling were done with minimal stress to the subjects. The blood volume of the sheep is sufficiently large to permit frequent collections of the many blood samples required in this investigation. Unlike plasma or
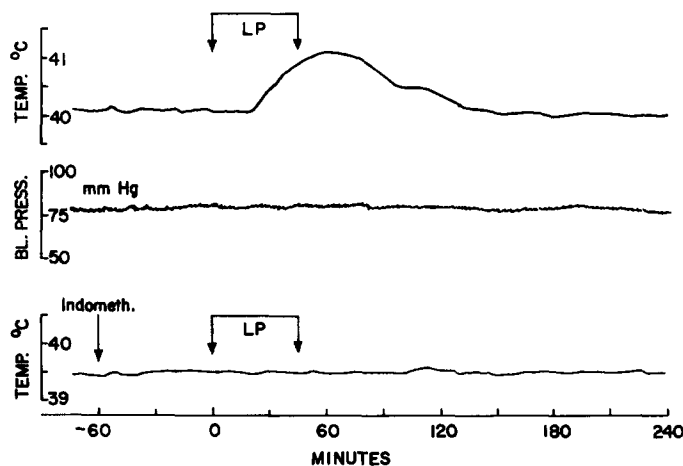

Fig. 7. Attenuation of the fever response to the intracarotid infusion of 25 ml of LP by pretreatment with indomethacin (4 mg/kg, subcutaneously).

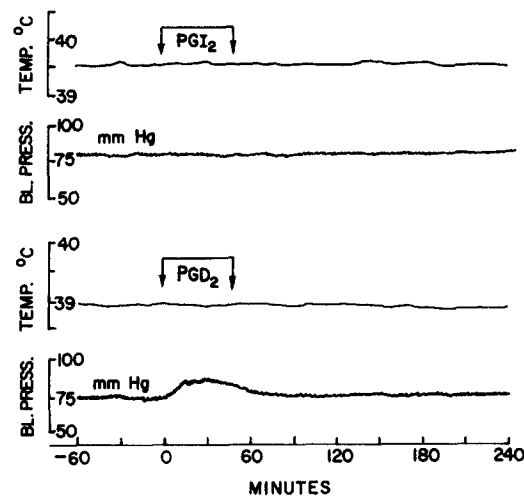Fig. 8. Effects of intracarotid infusions of PGI$_2$ (6.6 μg/min) or PGD$_2$ (2.6 μg/min) on body temperature and blood pressure.

serum from other mammalian species that we have studied (mouse, rat, rabbit, monkey, and human), ovine plasma was free of components that interfere with RIA, and the need to extract plasma samples was thereby avoided.

In taking simultaneous blood collections from the carotid artery and jugular vein during fever episodes, it was anticipated that plasma PG levels, if detectable, would show obvious arterio-venous differences across the brain during fever. This was not the case, however, in animals receiving 4–10 μg of endotoxin. In such experiments, arterial plasma contained nearly as much PG as plasma from the jugular vein. The presence of PG in carotid arterial plasma suggests that either PG synthesis (and release) by the lung is increased or that PG metabolism is compromised during the initial phase of fever. The latter possibility is more probable because of reports of a marked inhibition of PG metabolism in lung and liver preparations from endotoxin-treated animals (26, 27).

When animals were given infusions of <2 μg of endotoxin, significant arteriovenous differences in PG content of plasma were observed (Fig. 4). Little or no PGE was present in carotid plasma, whereas a surge of PGE appeared in jugular vein plasma coincident with the first fever peak and pressor response. These experiments imply that very small doses of endotoxin do not impair PG metabolism in the lung, and they suggest that the fever response can be initiated by a local synthesis and release of PG in the cranial vasculature. There is little doubt that with higher doses of endotoxin, the resultant surge of PGE from the carotid artery serves to magnify the early temperature rise and pressor effect.

Of the PG tested, only PGE$_2$ evoked a fever response when infused via the carotid artery. The specificity of PGE$_2$ as a pyrogen is striking considering the fact that the structurally similar PGD$_2$ compound did not produce fever, even though it appeared to be equipotent as a pressor agent. The abrupt pressor effect of intracarotid infusions of PGE$_2$ has been found to be centrally mediated (28), and it is probable that the early temperature and pressor responses to circulating endotoxin are also central effects mediated by PGE.

The close temporal relationship between the appearance of PGE in the circulation and the rapid onset of fever and pressor responses to endotoxin mirrored the responses obtained with intracarotid infusions of PGE_2. This observation implies that the early temperature and blood pressure responses to endotoxin are initiated by PGE generated in the blood compartment and that this PG diffuses rapidly across the blood-brain interface to act on appropriate receptors in the brain. In preliminary experiments (Kolb, Bovaird, and McCracken, unpublished results), PG has been identified by RIA in the CSF of the sheep within 3 min from the start of intracarotid infusions and in jugular vein plasma within 3 min of intraventricular administration of PG.

Although it was anticipated that indomethacin would attenuate the initial fever and pressor responses to endotoxin, we did not expect a total inhibition of fever because plasma RIA indicated the absence of PGE (and pressor effect) during the late phase. This finding is compatible, however, with the observed indomethacin block of the LP fever response which, according to our data, is not associated with circulating PGE. The work of Cranston and colleagues (13, 14) provides more direct evidence that PGE does not mediate LP fever, even when this pyrogen is administered via a lateral cerebral ventricle.

In the aggregate, our results implicate PGE as the mediator of the early fever response to circulating endotoxin and suggest that an additional pyrogenic derivative of arachidonate metabolism is responsible for the second phase of the biphasic response. Support for this view is found in the work of Laburn et al. (29), who showed that whereas indomethacin effectively diminished the temperature response to intraventricular injections of sodium arachidonate, PG antagonists attenuated only the early part of the fever response when coinjected with arachidonate.

The PGE associated with the initial fever response may originate, in part, from the macrophage because this cell has been shown to synthesize and release PGE when exposed to endotoxin in vitro (30). It is also conceivable that circulating LP provides an additional stimulus for pyrogen release from cells such as granulocytes, macrophages, and platelets, all of which are capable of producing primary PG, endoperoxides, and/or thromboxanes (31–36). Rather than assume a direct action of LP on thermoregulatory centers in the brain, we favor the thesis that the second phase of fever is, like the early phase, mediated by a pyrogenic derivative of arachidonate metabolism produced by cells within the blood compartment. Accordingly, the total biphasic fever response to endotoxin may reflect a sequential transport of pyrogenic metabolites across the blood-brain interface.

Summary

Biphasic fevers were induced in sheep with intravascular infusions or injections of 4–10 μg (80–200 ng/kg) of endotoxin, whereas monophasic fevers were obtained with doses of 1–2 μg (20–40 ng/kg). A marked increase in arterial blood pressure invariably accompanied the onset of fever; the latency of responses to the higher and lower doses of endotoxins averaged 26 min and 42 min, respectively. Prostaglandin (PG) assays of plasma from the carotid artery and jugular vein during fever episodes revealed a surge of PGE and PGF coincident with the pressor response and the first phase of fever, but PG were not detected in plasma samples taken throughout the second phase of fever. PG measurements of arterial and venous plasma collected at a distal site (hind limb) showed a similar surge of PGE and PGF in association with the early fever response,

indicating that intravascular PG synthesis and release represents a generalized systemic response to circulating endotoxin. Carotid arterial infusions of PGE_2 produced immediate monophasic fevers and pressor responses, whereas PGD_2 infusions produced an immediate pressor effect but no fever. Infusions of $PGF_{2\alpha}$ or prostacyclin, however, evoked neither fever nor pressor effects. Intracarotid infusions of leukocyte pyrogen (LP) caused monophasic fevers with latent periods of 15–20 min but pressor responses were not seen and neither PGE nor PGF were detected in plasma samples from the carotid artery or jugular vein before or during fever. Indomethacin, a potent inhibitor of arachidonic acid metabolism, blocked fever responses to endotoxin and to LP. These findings implicate PGE as the mediator of the early phase of endotoxin fever and imply a role for another pyrogenic metabolite of arachidonic acid in the mediation of the second phase of fever, i.e., the phase associated with circulating LP. It is possible that both pyrogenic metabolites are generated within the vascular compartment, reaching thermoregulatory centers of the brain by transfer across the blood-brain interface.

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