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Blood-borne, albumin-bound prostaglandin E_2 may be involved in fever

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Romanovsky, Andrej A., Andrei I. Ivanov, and Elena K. Karman. Blood-borne, albumin-bound prostaglandin E_2 may be involved in fever. *Am. J. Physiol.* 276 (*Regulatory Integrative Comp. Physiol.* 45): R1840–R1844, 1999.—Although the involvement of blood-borne PGE_2 in fever has been hypothesized by several authors and has substantial experimental support, the current literature often rejects this hypothesis because several attempts to induce fever by a peripheral PGE_2 failed. However, it is usually ignored that the amphipathic molecules of PGE_2 are readily self-associating and that such an aggregation could have prevented the peripherally administered PGE_2 (free form) from expressing its pyrogenic activity, thus leading to false negative results. To ensure disaggregation of PGE_2, we prepared its complex within a carrier protein, human serum albumin (HSA). HSA was purified with activated charcoal and polymixin B-polyacrylamide gel and incubated with PGE_2 for 1 h at 37°C. Adult Chinchilla rabbits were injected intravenously with PGE_2-HSA complex in either the higher (75 μg/kg PGE_2:30 mg/kg HSA) or the lower (15 μg/kg:6 mg/kg) dose, and the rectal temperature (T_r) was measured. In the controls, either the ligand alone or the carrier alone was administered. At the higher dose, neither free PGE_2 nor albumin alone was pyrogenic, whereas the PGE_2-HSA complex produced a fever characterized by a short latency (<10 min) and a maximal T_r rise of 0.7 ± 0.2°C. At the lower dose, none of the substances affected the T_r. This study demonstrates a marked pyrogenic activity of the intravenous PGE_2-HSA, but not of the free PGE_2. Administration of a preformed complex may be more physiologically relevant than administration of the free ligand because of the ligand's disaggregation, protection from enzymatic degradation, and facilitated delivery to targets. Our study supports the hypothesis that peripheral PGE_2 is involved in fever genesis.

prostanoids; carrier-mediated transport; febrile response; temperature regulation; neuroimmunomodulation; rabbits

SINCE MILTON AND WENDLANDT (19) discovered the pyrogenic effect of centrally injected PGs of the E series, PGE_2 has been considered an ultimate mediator of fever (2, 18, 42). However, it is still unclear whether the febrigenic PGE_2 is produced inside or outside the blood-brain barrier (BBB) (2, 42). The important role of peripheral PGE_2 in fever was originally proposed by Dascombe and Milton (7) in 1979 and restated in the 1980s by Skarnes et al. (41), Rotondo et al. (36), and Morimoto et al. (22). The febrigenic role of blood-borne PGE_2 has been supported by several lines of evidence. First, PGE_2 concentration in the venous blood of rabbits and sheep (20, 36, 41) and in the carotid blood of sheep (41) increases simultaneously with the onset of fever. Second, peripherally administered inhibitors of either the precursor release (18) or the synthesis of PGs (21, 36) block the febrile response even if they do not cross the BBB (rabbits). Third, as it has been shown in several species, intravenous PGE_2 crosses the BBB (1, 7, 11, 16) and produces dose-dependent fevers (11, 26, 40); the intracarotid PGE_2 is also highly pyrogenic in sheep (5, 41). However, there is also a great body of data contradicting the peripheral origin of febrigenic PGE_2 (for review, see Ref. 2). Thus several studies in rabbits, guinea pigs, and sheep that attempted to induce fever by intravenous (17, 19, 41) or intracarotid (20, 38) PGE_2 or PGE_1 failed, as did studies in rabbits and guinea pigs that attempted to detect PGE_2 in the cerebrospinal fluid (20) or preoptic microdialysate (38) after PGE_2 infusion into the carotid artery. As a result, the idea of blood-derived PGE_2 playing an important role in fever has been either ignored (37, 42) or rejected (2) by many authors, including some of the early proponents (20). In the present study, we suggest that such a rejection might have been premature and that the reported inability of peripheral PGE_2 to enter the brain and trigger the febrile response might have had a methodological explanation.

When PGs are administered peripherally, high-concentration ($\geq 10^{-3}$ M) aqueous solutions with a small amount of an organic solvent (usually ~1% ethanol) are commonly used (17, 19, 38). However, PGs are amphipathic, poorly water-soluble substances; at these concentrations, they are likely to self-aggregate as a result of interactions between the hydrophobic cyclopentane rings and/or numerous intermolecular hydrogen bonds (10). Such an aggregation is well known for changing the processes of body distribution and diminishing the specific biological activity of amphipaths (15). On the other hand, when aggregation of amphipathic substances is prevented (e.g., by administering them in lipid vesicles), their specific biological effects are easily revealed. Thus incorporation of PGE_1

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into soybean oil microspheres decreases the clearance of the PG from the blood and markedly potentiates its inhibition of platelet aggregation (24). Another way to reduce the aggregation of PGs in aqueous solutions is to decrease the PG concentration and/or increase the concentration of an organic solvent, such as ethanol. Interestingly, those studies demonstrating a pyrogenic effect of intravenously administered PGE_2 (11, 40) involved either a high concentration of ethanol (15%) or a low concentration of the PG (200 μM) in the injected solution, whereas the studies failing to induce fever by intravenous PGE (17, 19, 41) used a 30–75× lower concentration of ethanol (41), did not use ethanol at all (17, 19), or worked with PG concentrations up to 30× higher (17).

Yet another way to prevent aggregation of PGs is by binding to serum albumin, a principal transport protein for amphipathic molecules in blood (27). Based on in vitro binding studies, it has been suggested that up to 99% of the circulating PGE_2 is albumin bound (3, 45). If exogenous PGs administered in high-concentration aqueous solutions were to readily bind to blood albumin, there would be no concerns regarding their aggregation. However, the binding rate of exogenous PGE_2 to albumin is much lower than the rate of its clearance. Indeed, only 60% of PGE_2 is bound to albumin after a 5-min incubation in vitro (31), whereas, in vivo, >50% of a radiolabeled PGE_2 is cleared from the circulation within 30 s (13), presumably by the lungs (28). Therefore, a physiologically meaningful binding of an exogenous PGE_2 to albumin would require their joint preincubation.

In the present study, we preincubated PGE_2 with human serum albumin (HSA) to allow for the formation of a PGE_2-HSA complex. Low and high doses of both the free form and the HSA-bound form of PGE_2 were injected intravenously in rabbits, and their pyrogenic activities were compared.

METHODS

Animals. Twenty-nine male Chinchilla rabbits weighing ~3.3 kg were purchased from the Minsk Society of Rabbit Breeders (Minsk, Belarus). The animals were housed in individual cages and exposed to a 12:12-h light-dark cycle (lights on from 8:00 AM) and a neutral (23°C) ambient temperature. Food and water were available ad libitum. During the week preceding an experiment, the animals underwent at least three 4-h-long sessions of habituation to the experimental conditions. Immediately before an experiment, all the animals were deprived of food (but not water) for 12 h. To obviate the possible effects of circadian rhythms, all experiments were started around 10:00 AM. Each animal was used in an experiment only once and killed with an intravenous injection of pentobarbital sodium thereafter.

Drugs. HSA (Fraction V; Blood Transfusion Center, Vitebsk, Belarus) was processed to remove endogenous fatty acids, which are known to inhibit PGE_2 binding to albumin (6). HSA was dissolved in pyrogen-free water (50 mg/ml) and defatted according to Chen's method (4). This procedure resulted in a decrease of the molar ratio of nonesterified fatty acids (determined by Duncombe's method, Ref. 9) to HSA (bromcresol green dye assay, Ref. 8) from ~1.5 to <0.1. To eliminate potential lipopolysaccharide contamination, the defatted HSA was diluted twofold with pyrogen-free sodium chloride (1.8% wt/vol), alkalinized to pH 7.4, and gently stirred for 60 min at room temperature with Polymixin B (Sigma, St. Louis, MO) covalently attached to polyacrylamide beads according to the procedure of Plate et al. (29). The purified HSA was then passed through a sterile 0.22 μM Millipore filter (Sigma), frozen, and stored at −20°C. A complex of PGE_2 (Sigma) was freshly prepared each day by diluting its ethanol stock (3 mg/ml) with the purified HSA (20 mg/ml) and incubating it at 37°C for 1 h. The final concentrations of PGE_2, HSA, and ethanol in the incubate were 50 μg/ml, 20 mg/ml, and 1.7%, respectively. For control experiments, a solution of the free PGE_2 was prepared by diluting its ethanol stock with a pyrogen-free isotonic saline.

Experimental protocols. The pyrogenic activities of the free PGE_2, free HSA, and PGE_2-HSA complex were tested. The drug of interest was injected into a marginal ear vein, and the animal's rectal temperature (T_r) was measured with an electronic thermometer (Institute of Experimental Medicine, St. Petersburg, Russia) from 1 h before to 4 h after the injection; the probe length was 5 cm. Both the free and bound PGE_2 were injected in two doses: a low one (15 μg/kg of PGE_2 and 6 mg/kg of HSA) and a high one (75 μg/kg of PGE_2 and 30 mg/kg of HSA). For the low dose, the drugs were injected at 0.3 ml/kg; for the high dose, the volume of injection was 1.5 ml/kg. The two PGE_2 doses chosen fit in the lower 2% portion of the range (3–3,500 μg/kg) cumulatively covered by nine earlier studies (cited in introduction) of thermal effects of PGE (free form) administrated peripherally.

Data analysis. Data (means ± SE) are presented as a deviation of the T_r (ΔT_r) from its preinjection level (averaged over a 45-min period). For each response, we determined two measures: the maximal value of ΔT_r (ΔT_{rmax}) and the fever index (FI). The latter was calculated as an integral of the ΔT_r time function over 0–4 h postinjection. Each of the two obtained response measures (FI and ΔT_{rmax}) was subjected to a two-way ANOVA followed by Scheffé's test. To compare the urination- and defecation-inducing effects of the albumin-bound vs. the free form of PGE_2, the exact Wilcoxon rank-sum test was used.

RESULTS

The thermal responses of the rabbits to the low dose of HSA, PGE_2, and PGE_2-HSA are presented in Fig. 1*A*. At this dose, none of the drugs caused a fever. An ANOVA found no differences in the thermal responses (FI, ΔT_{rmax}) to the three treatments [FI, $F(2,13) = 0.21$, $P = 0.82$; ΔT_{rmax}, $F(2,13) = 0.29$, $P = 0.75$]. The thermal responses to the high dose of HSA, PGE_2, and PGE_2-HSA are presented in Fig. 1*B*. The results of an ANOVA of FI and ΔT_{rmax} were identical [$F(2,10) = 7.2$, $P = 0.01$], thus rejecting the hypothesis of the three responses being alike. Indeed, neither free PGE_2 nor HSA alone was pyrogenic, whereas the injection of the preformed PGE_2-HSA complex resulted in a marked fever. This fever had a very short (<10 min) latency, reached its maximum (0.7 ± 0.1°C) at 126 ± 28 min, and persisted over ~4 h (Fig. 1*B*). Scheffé's analysis showed that PGE_2-HSA-induced fever was significantly different from the thermal responses to both free PGE_2 (FI, $P = 0.02$; ΔT_{rmax}, $P = 0.01$) and HSA (FI, $P = 0.05$; ΔT_{rmax}, $P = 0.02$).

The rabbits receiving the high dose of PGE_2 (75 μg/kg) in the albumin-bound form responded to the

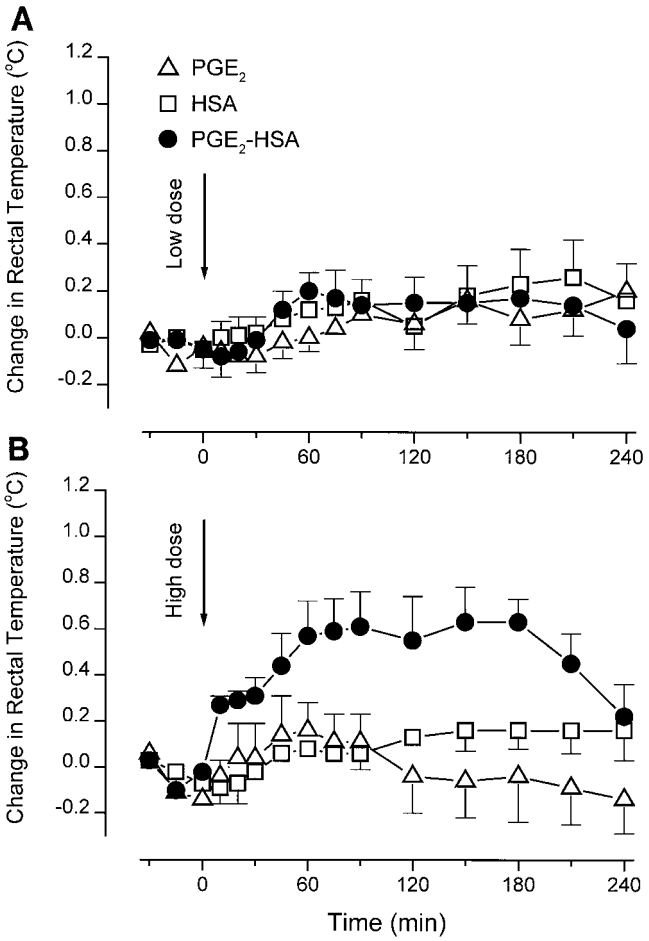

Fig. 1. Thermal responses of rabbits to intravenous injection (arrow) of preformed complex of PGE_2 with human serum albumin (HSA) at low (*A*) or high (*B*) dose. Control animals received either free form of PGE_2 or HSA alone. In low-dose experiment, drugs were injected as follows: free PGE_2 at 15 μg/kg (n = 5 animals), HSA at 6 mg/kg (n = 5), and PGE_2-HSA complex at 15 μg/kg:6 mg/kg (n = 6). In high-dose experiment, drugs were administered as follows: PGE_2 at 70 μg/kg (n = 4), HSA at 30 mg/kg (n = 4), or PGE_2-HSA at 70 μg/kg:30 mg/kg (n = 5). In all experiments, initial rectal temperature (T_r) was similar; mean initial T_r for whole study was 39.1 ± 0.1°C.

injection with rapid (1–3 min postinjection) defecation (100% of the animals) and urination (60%), whereas the animals treated with the free form exhibited both symptoms later (>3 min; P = 0.15) and somewhat less regularly (defecation, 75%; urination, 50%). [Interestingly, a smaller dose of free PGE_2 (~45 μg/kg, intracarotid) in lambs caused defecation only occasionally (5).] No urination or defecation at all was detected during the first 10 min after PGE_2 administration at the low dose (15 μg/kg), whether free or HSA bound, or after HSA injection at any dose.

DISCUSSION

Pyrogenic activity of albumin-bound PGE_2. The present study demonstrates a marked pyrogenic activity of PGE_2 when it is administered intravenously in a preformed complex with HSA, but not when it is given in the free form. Why is binding to albumin required for the pyrogenic activity of intravenous PGE_2? Serum albumin is a principal carrier in the blood of various amphipathic organic molecules (27), including PGs (3, 31, 45). Adding albumin to an aqueous solution of an amphipathic ligand results in a left shift in the equilibrium: albumin-bound ligand ↔ monomeric ligand ↔ self-aggregated ligand. As the result of this shift, the ligand's biological activity may be affected in three ways. First, a larger proportion of the aggregated ligand becomes transferred into the monomeric form, which is the most biologically active form (15). Second, a larger proportion of the monomeric ligand becomes bound to albumin; this may of itself enhance delivery of the ligand to receptors on target cells and facilitate its cellular influx, as has been demonstrated for various amphipaths, including long-chain fatty acids (30) and bilirubin (25). An enhancing effect of albumin on PGE_2 binding to a membrane receptor has also been reported (6). Third, the increased proportion of the albumin-bound fraction makes the ligand less accessible for degrading enzymatic systems; this is certainly true for PGs (14).

It was suggested by Székely and Szelényi (43) that exogenous (free, self-aggregated?) PGE may not act in exactly the same way as the PG liberated endogenously (bound to albumin?) during fever; this argument was used to explain some contradictory data on the role of PGs in the febrile response. We speculate that administration of the albumin-bound PGE_2 is more physiologically relevant than administration of the free form. As our data clearly demonstrate, the biological effects of the two forms may be different. Thus the effect of PGE_2 on smooth musculature (urination and defecation) occurred significantly earlier when the drug was administered in the preformed complex with HSA. More importantly, the bound form of PGE_2 was febrigenic, whereas the free PGE_2 was not. A peripheral administration of free PGs in aqueous solutions is likely, therefore, to lead to false negative results. If so, some earlier attempts to induce fever via the peripheral administration of free PGs might have been unsuccessful as a result of this exact reason. Nevertheless, the negative results obtained in these earlier studies led to the dismissal of any important role of blood-borne PGE_2 in fever (2, 20). We suggest that the validity of such a dismissal should be questioned, and the physiological role of blood-borne PGs should be revisited.

Peripheral PGE_2 in fever. The pyrogenic activity of intravenous PGE_2 (bound form) is in line with the data of others, who showed that intracarotid (5, 41) or intravenous (1, 7, 11, 16, 26, 40) PGE_2 successfully reaches the brain and induces a dose-dependent fever. Thus our study provides additional support to the intriguing hypothesis of blood-borne PGE_2 playing a role in fever (7, 18, 22, 36, 41). Yet, the mechanisms of the participation of blood-borne, albumin-bound PGE_2 in fever remain speculative. A priori, three possibilities can be suggested. First, binding of the PGE_2-albumin complex to brain endothelial cells (via either a specific interaction with the albumin-binding protein or a nonspecific interaction with the lipid bilayer of the membrane) may facilitate dissociation of the complex (32,

44), thus facilitating PGE_2 uptake by the cells. From there, PGE_2 may either further diffuse into the neural tissue or trigger the synthesis and/or release of other pyrogenic mediators in situ. Second, PGE_2 may codiffuse with albumin in the perivascular space in the periventricular organs (such as the organum vasculosum laminae terminalis), dissociate at the surface of neurons or nonneural cells, and thus trigger the febrile response either directly or via the synthesis and/or release of pyrogenic mediators. Indeed, albumin is known to easily penetrate the fenestrated endothelium of various vascular beds and enter the tissue; in fact, the total extravascular pool of albumin is two times larger than the total intravascular pool (27). In the brain, such a penetration allows blood albumin to reach the cerebrospinal fluid; 80% of all protein in the cerebrospinal fluid is albumin (27). Third, afferent fibers of peripheral nerves may constitute another target for the albumin-bound PGE_2. Morimoto et al. (21) have hypothesized that peripheral nerves play an important role in the genesis of the early febrile phase. (The term early febrile phase refers to the only phase of a monophasic fever or to the first phase of a polyphasic febrile response; see Ref. 33). Recently, we have shown that the development of the early phase of lipopolysaccharide fever requires the integrity of the vagus nerve (35) and, specifically, its hepatic branch (39). On the other hand, blood-borne PGE_2 is also considered to be a mediator of the early febrile phase (18, 22, 41). Furthermore, vagal sensory neurons express PG receptors; peripheral PGE_2 exhibits multifaceted regulatory actions by modifying vagal afferent transmission (for review, see Ref. 12); and blood-borne (not synthesized in situ) albumin is present in peripheral nerves (27). Finally, it has been recently shown that activation of vagal afferents by cytokines is PG mediated (12, 23). Although further research is needed to determine a partial contribution of each of these three putative mechanisms of blood-borne, albumin-bound PGE_2 to the genesis of the fever response, some speculation is natural.

Perspectives

We speculate that blood-derived PGE_2 carried by serum albumin is likely to mediate the early febrile phase through an action on neural afferent fibers, perhaps of the hepatic vagal branch. Because this phase of fever can be blocked by truncal vagotomy (35), hepatic branch transection (39), capsaicin desensitization of intra-abdominal afferents (M. Székely and M. Balaskó, unpublished), and peripheral administration of PG synthesis inhibitors that do not cross the BBB (18, 21, 36), the proposed mechanism is likely to constitute the major mechanism of the early phase. As for the later febrile phases (second, third, etc.; see Ref. 34), their genesis is unlikely to involve afferent fibers of peripheral nerves (35). Therefore, if PGs are mediators of the later fever (as has been proposed; see Refs. 18, 22), they act on different targets, perhaps those located in either the perivascular space of the circumventricular organs or the neural tissue behind the BBB (2, 37, 42). In either case, these targets may be accessible to both blood-derived PGs and those synthesized locally. Thus the proposed schema of the febrile pathogenesis incorporates both the blood-borne and the brain-borne PGs. The question of whether the febrigenic PGs are of the peripheral or central origin should probably be rephrased to which stages of febrile pathogenesis are mediated by the peripheral PGs and which stages by the central?

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