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Albumin is not an irreplaceable carrier for amphipathic mediators of thermoregulatory responses to LPS: compensatory role of α_1-acid glycoprotein

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Ivanov, Andrei I., Alexandre A. Steiner, Shreya Patel, Alla Y. Rudaya, and Andrej A. Romanovsky. Albumin is not an irreplaceable carrier for amphipathic mediators of thermoregulatory responses to LPS: compensatory role of α_1-acid glycoprotein. *Am J Physiol Regul Integr Comp Physiol* 288: R872–R878, 2005. First published December 2, 2004; doi:10.1152/ajpregu.00514.2004.—In view of the potential involvement of peripherally synthesized, circulating amphipathic mediators [such as platelet-activating factor (PAF) and prostaglandin E_2] in the systemic inflammatory response to lipopolysaccharide (LPS), we hypothesized that transport of amphipaths by albumin is essential for conveying peripheral inflammatory signals to the brain. Our first specific aim was to test this hypothesis by studying LPS-induced fever and hypothermia in Nagase analbuminemic rats (NAR). NAR from two different colonies and normalbuminemic Sprague-Dawley rats were preimplanted with jugular catheters, and their febrile responses to a mild dose of LPS (10 μg/kg iv) at thermoneutrality and hypothermic responses to a high dose of LPS (500 μg/kg iv) in the cold were studied. NAR of both colonies developed normal febrile and hypothermic responses, thus suggesting that transport of amphipathic mediators by albumin is not indispensable for LPS signaling. Although alternative carrier proteins [such as α_1-acid glycoprotein (AGP)] are known to assume transport functions of albumin in NAR, it is unknown whether inflammatory mediators are capable of inducing their actions when bound to alternative carriers. To test whether PAF, the most potent amphipathic pyrogen, causes fever when administered in an AGP-bound form was our second aim. Sprague-Dawley rats were preimplanted with jugular catheters, and their thermal responses to infusion of a 1:1 [PAF-AGP] complex (40 nmol/kg iv), AGP (40 nmol/kg iv), or various doses of free (aggregated) PAF were studied. The complex, but neither free PAF nor AGP, caused a high (~1.5°C) fever with a short (<10 min) latency. This is the first demonstration of a pyrogenic activity of AGP-bound PAF. We conclude that, in the absence of albumin, AGP and possibly other carriers participate in immune-to-brain signaling by binding and transporting amphipathic inflammatory mediators.

systemic inflammation; fever; hypothermia; body temperature; platelet-activating factor; Nagase analbuminemic rats

BACTERIAL LIPOPOLYSACCHARIDE (LPS) is widely used to induce systemic inflammation and its thermoregulatory manifestations, that is, fever and hypothermia, in laboratory animals. In the rat, an intravenous dose of LPS administered at a neutral or supraneutral ambient temperature (T_a) typically causes fever (51, 52, 55, 63). When T_a is subneutral, intravenous LPS evokes fever at low doses, mild hypothermia followed by fever at intermediary doses, and pronounced hypothermia at high, shock-inducing doses (52, 53, 55, 63). Both the febrile and hypothermic responses to LPS are mediated by bioactive lipids, such as platelet-activating factor (PAF), prostaglandins (PG) E_2 and D_2, and leukotrienes (22, 24, 46, 68). Peripherally synthesized, blood-borne pools of these lipids are thought to play a role in conveying febrigenic and hypothermic signals to the brain; such a role is best documented by the mediation of LPS fever by circulating PGE_2 (for review, see Ref. 24). First, the earliest (first) phase of LPS fever in rats is accompanied by massive expression of PGE_2-synthesizing enzymes in the liver and in lungs but not in the brain (23). Second, PGE_2 concentration in the venous blood of rabbits and sheep (37, 57, 61) and in the carotid blood of sheep (61) increases simultaneously with the onset of fever. Third, peripherally administered inhibitors of PG synthesis (38, 57), inhibitors of the release of a PG precursor (see Ref. 35), and antibodies to PGE_2 (Steiner AA, Ivanov AI, Rudaya AY, Dragic AS, and Romanovsky AA, unpublished observations) block the febrile response even if they do not cross the blood-brain barrier. Fourth, intravenous and intracarotid PGE_2 have been repeatedly shown to enter the brain (2, 6, 8, 27) and produce dose-dependent fevers (4, 8, 45, 49, 60, 61) in several animal species.

In the blood, amphipathic lipid mediators, including PGE_2 and PAF, exist predominantly as complexes with their principal carrier protein, albumin (31, 48, 69). In the free form, these lipids are poorly soluble in water and readily self-aggregate (7, 28). Binding to albumin typically enhances biological activity of an amphipathic mediator by shifting the equilibrium from the free, aggregated (less biologically active) form to the bound, monomeric (most active) form; it also protects the mediator from rapid degradation by extracellular enzymes (13, 47). In the case of PGE_2 or PAF, intravenous infusion of an albumin complex with either mediator produces a more pronounced febrile response at a neutral T_a than infusion of the same mediator in its free (aggregated) form (22, 49). Similarly, intravenous PAF produces a more pronounced hypothermic response at a subneutral T_a when administered as a [PAF-albumin] complex than when infused in the free form (22). In view of the potential involvement of peripherally synthesized, circulating amphipaths in the systemic inflammatory response to LPS, we hypothesized that binding of amphipathic mediators to and their transport by albumin are essential for conveying peripheral inflammatory signals to the brain and, therefore, for normal development of the inflammatory response. The

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first specific aim of the present study was to test this hypothesis by studying LPS-induced fever and hypothermia in Nagase analbuminemic rats (NAR; see Refs. 40, 41).

These Sprague-Dawley (SD)-derived rats produce virtually no albumin due to a mutation in the albumin gene; this mutation results in transcription of defective mRNA (10, 59). The lack of albumin in NAR readily reveals itself by altered pharmacokinetics (increased free fraction, accelerated clearance) of some exogenous amphipathic drugs (15, 16, 19, 39, 42). However, the functionally verifiable lack of albumin in NAR causes relatively few and relatively mild side effects. Due to multiple compensatory mechanisms, these rats have essentially normal content of total protein in the plasma, colloid osmotic pressure, blood pressure, and hepatic and renal functions (17, 25, 26, 36, 39). This makes NAR a unique, reasonably pathology-free model to study the role of albumin. However, the other side of the issue is that the same compensatory mechanisms may mask the effect of analbuminemia on the transport of the mediator studied, even if this mediator is normally carried by albumin, whether exclusively or predominantly. Hence, negative results obtained in NAR are difficult to explain, and the potential compensatory mechanisms should be considered.

An important compensatory role in analbuminemia is played by α_1-acid-glycoprotein (AGP), a 44-kDa glycoprotein, considered the next important carrier for circulating amphipaths after albumin (20). Because AGP binds (although with a lower affinity) many amphipathic mediators that are normally bound to albumin, it "automatically" becomes a principal carrier of these mediators in the absence of albumin (29, 47), even if the concentration of AGP does not increase. For those cases of analbuminemia when the concentration of AGP increases (for review, see Ref. 17), binding to AGP becomes an even more powerful compensatory mechanism. Importantly, AGP is known to bind PAF, the most proximal mediator of LPS action (30) and the most potent amphipathic pyrogen (22). In an aqueous solution containing a physiological concentration of AGP (25 μM) and no albumin, ~99% of PAF is AGP bound (32, 33). Although AGP is known to assume transport functions of albumin in NAR (20), it is unknown whether inflammatory mediators are capable of inducing their actions when bound to this alternative carrier. We hypothesized that binding to AGP preserves the pyrogenic activity of PAF, and to test this hypothesis was the second specific aim of the present study. This aim was met by studying the febrile responses of normalbuminemic SD rats to free and AGP-bound PAF.

METHODS

Animals

Male SD-derived NAR were obtained from two different colonies: from the Utrecht University (Utrecht, The Netherlands; NAR/SaU) and from SLC (Hamamatsu, Japan; NAR/Slc). Wild-type SD rats were purchased from Harlan (Indianapolis, IN). All rats weighed 260–320 g at the time of the experiments. Initially, the rats were housed three per standard "shoe box"; after surgery, they were caged individually. The cages were kept in a rack equipped with a Smart Bio-Pack ventilation system (model SB4100) and Thermo-Pak temperature control system (model TP2000; Allentown Caging Equipment, Allentown, NJ); the temperature of the incoming air was maintained at 28°C. Standard rat chow (Teklad Rodent Diet "W" 8604; Harlan Teklad, Madison, WI) and tap water were available ad libitum. The room was on a 12:12-h light-dark cycle (lights on at 7:00 AM). 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 (7 daily training sessions, 4 h each). The same confiners were used later in the experiments. Rodents are readily adaptable to restraint to an extent that they respond to it with neither stress fever (54) nor other signs of stress (1, 12, 34, 62). Each rat was used in an experiment once. The rats were euthanized with pentobarbital sodium (20 mg/kg iv). The protocols were approved by the St. Joseph's Hospital Animal Care and Use Committee.

Surgery

On *day 0*, each rat was subjected to jugular catheterization under ketamine-xylazine-acepromazine (55.6, 5.5, and 1.1 mg/kg, respectively, ip) anesthesia and antibiotic (1.2 mg/kg sc enrofloxacin) protection. The animal was placed on an operating board, and a 1-cm longitudinal incision was made on the ventral surface of the neck, 1 cm left of the trachea. The muscles were retracted, and the left jugular vein was exposed, freed from its surrounding connective tissue, and ligated. A silicon catheter (0.5 mm ID, 0.9 mm OD) filled with heparinized (50 USP units/ml) pyrogen-free saline was passed into the superior vena cava through the jugular vein and secured in place with ligatures. The free end of the catheter was knotted, tunneled under the skin, and exteriorized at the nape. The surgical wounds were sutured. On *days 1* and *3* postsurgery, the catheter was flushed with heparinized (50 USP units/ml) saline.

Instrumentation

The experiments were performed on *day 5*. The animals were instrumented between 8:00 and 9:00 AM. For *experiment 1*, each rat was placed in a confiner and equipped with a copper-constantan thermocouple (Omega Engineering, Stamford, CT) to measure colonic temperature (T_c). This colonic thermocouple was inserted 9–10 cm beyond the anal sphincter. For *experiment 2*, each rat was additionally instrumented with a second copper-constantan thermocouple used to record tail skin temperature (T_{sk}). This was done to increase the chance of revealing thermoregulatory responses in this experiment. Indeed, changes in T_{sk} are often easier to detect than changes in T_c (52) because T_{sk} has a much wider operational range (50). The skin thermocouple was positioned on the lateral surface of the tail (at the boundary of the proximal and middle thirds) and fixed in place with adhesive tape. The thermocouples were plugged to a data logger (model AI-24; Dianachart, Rockaway, NJ) connected to a personal computer. The rat was transferred to a climatic chamber (Forma Scientific, Marietta, OH) set to either a subneutral (20.0°C) or neutral (30.0°C) T_a (50). The jugular catheter was extended with a length of PE-50 tubing filled with saline, and the extension was passed through a wall port and connected to a syringe filled with the drug of interest. The syringe was kept outside the chamber. This setup permitted injection of the drug without disturbing the rat. The instrumented rats were allowed to rest for at least 2 h before drug administration.

Drugs

All drugs and reagents were purchased from Sigma-Aldrich (St. Louis, MO). A stock suspension of *E. coli* 0111:B4 LPS (2.5 mg/ml) in saline was stored at −20°C. At the time of the experiment, the stock was diluted to a final concentration of either 10 μg/ml to induce fever or 500 μg/ml to cause hypothermia (*experiment 1*). To prepare a [PAF-AGP] complex for *experiment 2*, an ethanol solution of PAF (β-acetyl-γ-*O*-alkyl-L-α-phosphatidylcholine, from bovine heart lecithin; 2 μmol/ml) and a saline solution of bovine serum AGP (1 μmol/ml) were used as stocks (stored at −80°C and −20°C, respectively). On the day of the experiment, an aliquot (20 μl) of the ethanol stock of PAF (40 nmol) was dried in a SpeedVac centrifuge (model

SVC-200H; Thermal Savant, Holbrook, NY). The dried PAF was suspended in saline (0.4 ml), and the AGP stock and saline were added to the suspension to achieve final concentrations of PAF and AGP of 40 nmol/ml each. The same molar concentrations of PAF and AGP were used because PAF binds to a single major site in the AGP molecule (32, 33). The PAF-AGP-saline system was sonicated for 3 min and incubated for 1 h at 37°C. The incubation conditions were identical or similar to those used by others and by us in the past to prepare a [PAF-albumin] complex (22) and AGP complexes with various amphipaths (44, 70). In addition to the [PAF-AGP] complex, a solution of AGP alone (40 nmol/ml) and a suspension of free (aggregated) PAF (40, 80, or 160 nmol/ml) were used in *experiment 2*. The solution of AGP and the suspension of free PAF were sonicated and incubated exactly as the solution of the [PAF-AGP] complex. The higher concentrations of free PAF in the suspension were used because the loss of PAF during the incubation and intravenous infusion due to the adherence to plastic and glass is up to four times higher for a suspension of free PAF than for a solution of PAF in a protein-bound form (22).

Experimental Protocols

Experiment 1: LPS-induced fever and hypothermia in NAR. The febrile and hypothermic responses to LPS of NAR/SaU, NAR/Slc, and wild-type SD rats were studied. To induce fever, LPS was injected intravenously at a moderate dose of 10 μg/kg at a neutral T_a (30°C). Hypothermia was induced by a high dose (500 μg/kg iv) of LPS at a subneutral T_a (20°C). At either T_a, the controls were injected with saline (1 ml/kg iv). Six rats from each colony were allowed to recover from the experiment for 1 wk and used for verification of albumin content in their blood plasma (see below).

Experiment 2: thermal effect of a [PAF-AGP] complex. At T_a of 30°C, the SD rats were infused with the [PAF-AGP] complex (40 nmol/kg iv), AGP alone (40 nmol/kg iv), or free (aggregated) PAF (40, 80, or 160 nmol/kg iv). All infusions were performed at a rate of 1 $ml \cdot kg^{-1} \cdot h^{-1}$ over 1 h.

Verification of Analbuminemia of NAR

The absence of albumin in the plasma of the NAR/SaU and NAR/Slc and its presence in the plasma of SD rats were confirmed by electrophoresis. The rats were anesthetized with a small dose of ketamine-xylazine-acepromazine cocktail (5.56, 0.55, and 0.11 mg/kg, respectively, iv), and the arterial blood (3 ml) was collected by cardiac puncture with the use of a sterile syringe. The blood was immediately transferred to a Vacutainer tube (Becton Dickinson, Franklin Lakes, NJ) containing 100 USP units of sodium heparin. The plasma was separated by centrifugation (3,000 *g*, 10 min) at 4°C and stored at −80°C. The albumin level in the plasma was determined by electrophoresis in agarose gel. The separated proteins were visualized by Amido Black 10B staining.

Data Processing and Analysis

The absolute value of T_c, rather than the change in T_c, was used to evaluate deep body temperature responses in *experiments 1* and *2* (for justification, see Ref. 56). The heat loss index (HLI) was used to evaluate thermoeffector responses of tail skin vasculature in *experiment 2*. As justified elsewhere (50), the HLI was calculated according to the formula: $HLI = (T_{sk} - T_a)/(T_c - T_a)$. The theoretical limits of the HLI are 0 (maximal skin vasoconstriction) and 1 (maximal vasodilation). In practice, however, the upper limit depends on the position of the tail skin thermocouple. When T_{sk} is measured at the boundary of the proximal and middle thirds of the tail, as in the present study, the HLI rarely exceeds 0.6. The T_c and HLI responses were compared across treatments and time points by a two-way ANOVA for repeated measures. Tukey's (honest significant difference) post hoc test was used to determine whether there was a significant change in T_c or HLI over time ("effect of time") and whether there was a significant intertreatment difference in a T_c or HLI response ("effect of treatment"). The analysis was performed with Statistica AX'99 (StatSoft, Tulsa, OK). The effects were considered significant when $P < 0.05$. The data are reported as means ± SE.

RESULTS

Experiment 1: LPS-induced Fever and Hypothermia in NAR

At a neutral T_a of 30°C, the SD rats responded to a moderate dose of LPS (10 μg/kg iv) with a typical polyphasic fever ($P < 0.0001$, effect of time). As reported in previous studies (e.g., Ref. 54), three consecutive febrile phases peaked at ~60, 150, and 320 min postinjection (Fig. 1*A*). Febrile responses of similar duration and magnitude were observed in both colonies of analbuminemic rats, NAR/SaU (Fig. 1*B*) and NAR/Slc (Fig. 1*C*). A peculiar characteristic of the febrile responses of both NAR/SaU and NAR/Slc was the absence of a clear transition between the second and third febrile phases. In all rats studied, injection of saline was thermally ineffective (Fig. 1).

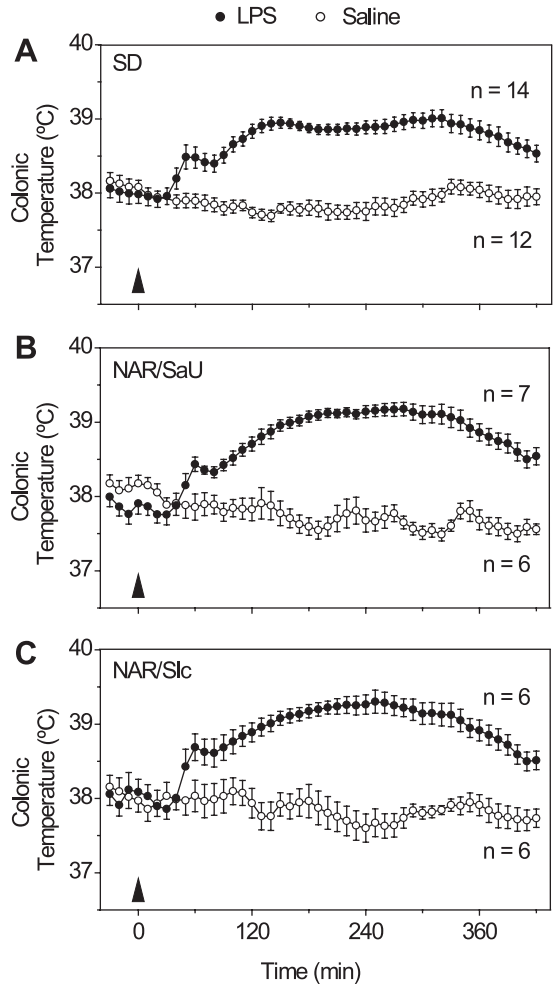

Fig. 1. Effects of intravenous injection (arrowhead) of lipopolysaccharide (LPS; 10 μg/kg) or saline (1 ml/kg) on the colonic temperature of normalbuminemic Sprague-Dawley (SD) rats (*A*), Nagase analbuminemic rats from Utrecht University (NAR/SaU; *B*), and Nagase analbuminemic rats from SLC (NAR/Slc; *C*). Experiments were conducted at a neutral ambient temperature of 30°C.

At a subneutral T_a of 20°C, the SD rats responded to a high dose of LPS (500 μg/kg iv) with a marked hypothermia ($P < 0.0001$, effect of time). As seen in previous studies (e.g., Ref. 55), this response had a latency of ~40 min and reached its nadir ~110 min postinjection (Fig. 2*A*). The hypothermic responses of either colony of NAR [NAR/SaU (Fig. 2*B*) and NAR/Slc (Fig. 2*C*)] did not differ from the response of the SD rats. Treatment with saline produced no thermal effect (Fig. 2).

The absence of albumin in NAR/SaU and NAR/Slc was confirmed by comparing the electrophoretic profiles of their plasma proteins with the profile of the SD rats (Fig. 3). Three main protein fractions corresponding to albumin, α-globulins, and β-globulins (40, 41) were robust in the SD rats. In the NAR of either colony, albumin was missing, but both α- and β-globulins were overexpressed. The overexpression was marked in the case of α-globulins and marginal in the case of β-globulins.

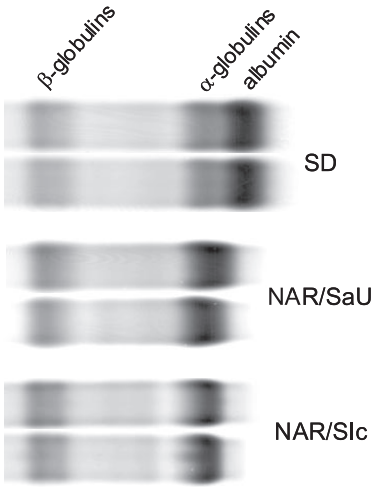

Fig. 3. Representative electrophoretograms (2 for each colony) of plasma proteins of SD rats (*top*), NAR/SaU (*middle*), and NAR/Slc (*bottom*). Fractions of interest are marked.

Experiment 2: Thermal Effect of a [PAF-AGP] Complex

At thermoneutrality, intravenous infusion of the [PAF-AGP] complex (40 nmol/kg) in the SD rats caused a fever characterized by a short (<10 min) latency and high (~1.5°C) magnitude ($P < 0.0002$, effect of time; Fig. 4*A*). A significant ($P < 0.004$, effect of time) decrease in the HLI (indicates tail skin vasoconstriction) paralleled the febrile rise in T_c (Fig. 4*A*). AGP alone tended ($P < 0.2$) to cause a slow (latency, ~40 min), small (magnitude, ~0.5°C) rise in T_c and produced weak vasoconstriction ($P < 0.014$, effect of time; Fig. 4*B*), possibly due to the immunogenicity of bovine AGP in the rat or a contamination of the 99% purity AGP preparation used with pyrogenic substances. When infused at a dose of 40 nmol/kg, free PAF was thermally ineffective (Fig. 4*C*) and so was saline (data not shown). However, to accurately compare effects of the [PAF-AGP] complex with those of free PAF, one should account for the fact that free PAF more readily binds to plastic and glass surfaces than protein-bound PAF. In the experimental paradigm used, the amount of PAF actually received by the animal is up to four times higher in the case of the protein-bound form than the free form (22). Hence, two and four times higher doses of free PAF (that is, 80 and 160 nmol/kg) were also used in the present work. At 80 nmol/kg, free PAF was still less effective than the 40-nmol/kg dose of the [PAF-AGP] complex ($P < 0.017$, effect of treatment): free PAF caused a very slow (latency of ~90 min), small (magnitude of ~0.5°C) T_c rise accompanied by vasoconstriction (Fig. 4*D*). Infusion of the highest dose of free PAF (160 nmol/kg) caused death of all the rats at ~50 min (Fig. 4*E*), thus indicating that the amount of PAF delivered was higher than the amount delivered by infusing the [PAF-AGP] complex (which induced no mortality). However, the fever response to even such a high, lethal dose of free PAF was still substantially lower (maximal rise ~0.5°C; $P < 0.0005$, effect of treatment) than that to the [PAF-AGP] complex.

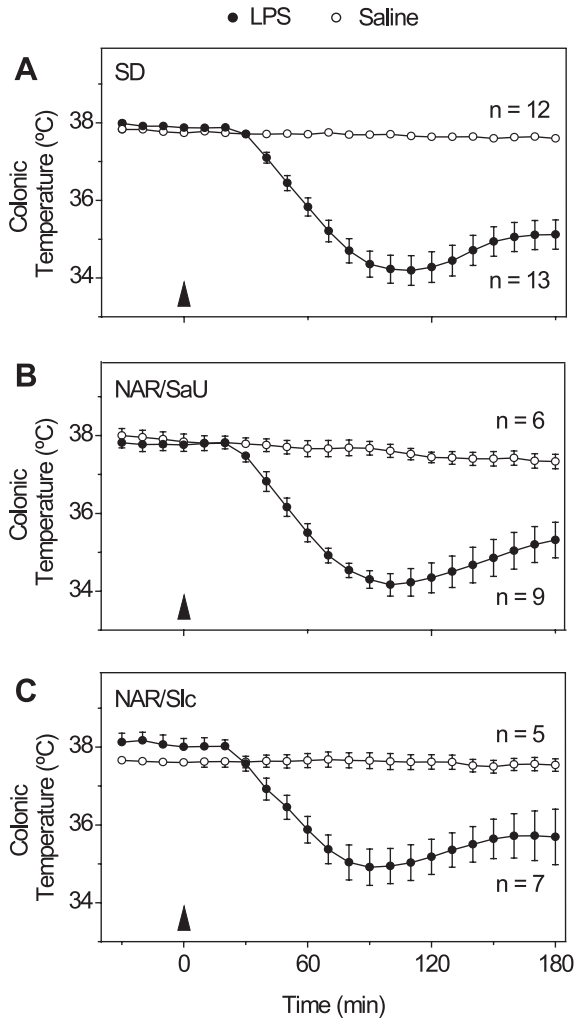

Fig. 2. Effects of intravenous injection (arrowhead) of LPS (500 μg/kg) or saline (1 ml/kg) on the colonic temperature of SD rats (*A*), NAR/SaU (*B*), and NAR/Slc (*C*). Experiments were conducted at a subneutral ambient temperature of 20°C.

DISCUSSION

The first aim of this study was to test whether transport of amphipathic inflammatory mediators by albumin is essential for development of the systemic inflammatory response to LPS. We addressed this aim by studying LPS-induced fever and hypothermia in NAR, a conventional animal model of

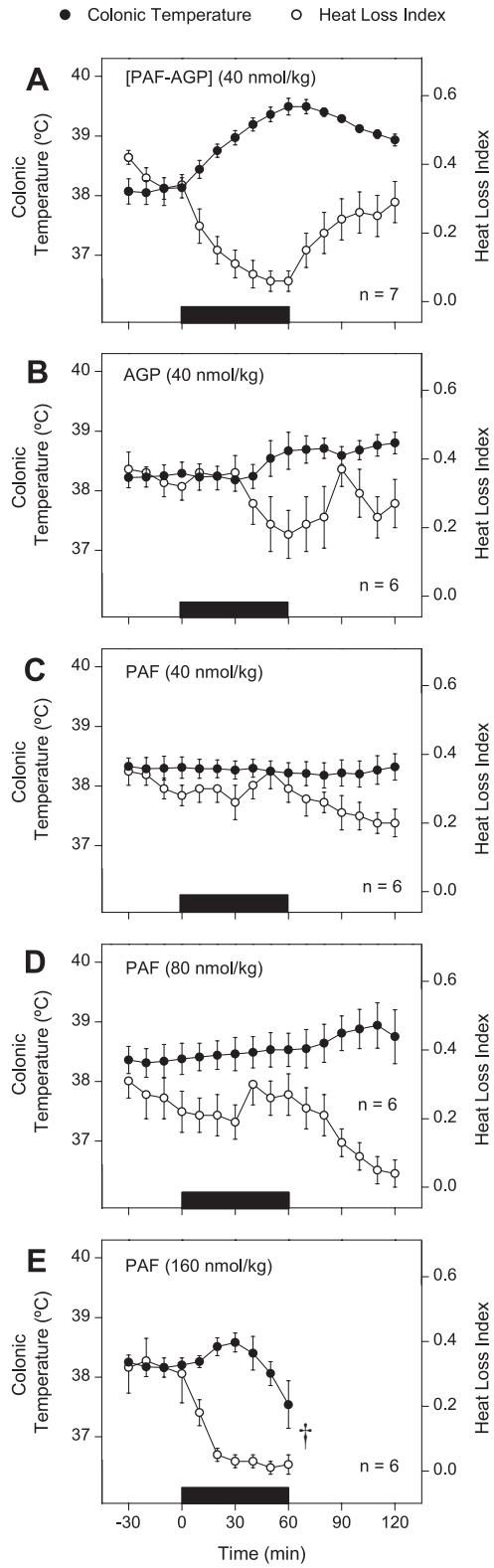

Fig. 4. Effects of intravenous infusion (solid bar) of the following substances on the colonic temperature (*left* ordinate axis) and heat loss index (HLI, *right* ordinate axis) of SD rats: [PAF-AGP], a 1:1 preformed complex of platelet-activating factor (PAF) and α_1-acid glycoprotein (AGP) at a dose of 40 nmol/kg (*A*); AGP, 40 nmol/kg (*B*); free (aggregated) PAF, 40 nmol/kg (*C*); free PAF, 80 nmol/kg (*D*); and free PAF, 160 nmol/kg (*E*). At the highest dose, free PAF caused death (†) of all rats at ~50 min after the beginning of infusion. The experiments were conducted at a neutral ambient temperature of 30°C.

albumin depletion (40, 41). We have found that both the febrile and hypothermic responses to LPS are normal in NAR, thus indicating that transport of peripherally synthesized, amphipathic mediators by albumin is not essential for LPS-induced thermoregulatory responses. These results have a twofold explanation. The first explanation is that peripherally synthesized, amphipathic mediators of LPS action normally carried by albumin are unimportant for the thermoregulatory responses to LPS. However, this possibility is highly improbable because it contradicts the large body of experimental evidence, suggesting a crucial importance of the circulating pool of at least one of these mediators, PGE_2, in at least one of the thermoregulatory responses, fever (see the Introduction). The second, more likely, explanation is that peripheral amphipathic mediators of LPS action are important, but that they are bound to and transported by alternative carriers in NAR.

Albumin binds the majority of ligands with a moderate to low affinity characterized by dissociation constants in a micromolar to millimolar range (47). Because such binding is less specific than a typical ligand-receptor interaction, other carrier proteins can often replace albumin in transporting its customary ligands (14, 21). In the absence of albumin (as happens in NAR), a customary ligand binds to the protein characterized by the most favorable combination of the binding constant and plasma concentration. Such compensation can be strictly functional, i.e., an alternative carrier can pick up the albumin's ligand without being overexpressed. Compensation can also involve overexpression of the alternative carrier; the large list of proteins overexpressed in the absence of albumin (see Refs. 3, 11, 17) includes α- and β-globulins, as seen in the present study (Fig. 3).

Functions that normally depend on transport of an amphipathic substance by albumin are compensated in NAR both functionally and expressionally. For example, pharmacokinetics of tryptophan (normally transported by albumin) are unaltered in NAR because tryptophan binds to and is carried by α_2-macroglobulin in these rats (9). The level of α_2-macroglobulin is increased in NAR (11); this is an example of expressional compensation. Plasma binding of bilirubin and its delivery to the liver are also normal in NAR (18, 72), possibly due to the bilirubin-binding property of high-density lipoproteins (65). However, this is a purely functional mechanism, as the level of high-density lipoproteins is not increased (and is even decreased) in NAR (see Ref. 17). For other examples of transport of amphipathic ligands by alternative carriers in hypoalbuminemia, see Refs. 9, 14, 21, 65, and 67.

AGP is considered the next important carrier for circulating amphipaths after albumin (20). Although the binding profiles of albumin and AGP are not identical (14, 21), they overlap substantially, and under conditions of analbuminemia AGP carries many amphipathic mediators normally carried by albumin (29, 47). Because the plasma concentration of AGP either remains unchanged (11) or increases (17) in analbuminemia, AGP is likely involved in both functional and expressional compensation. That amphipathic inflammatory mediators (e.g., PAF) bind to AGP in the absence of albumin is known (32, 33). It is unknown, however, whether binding to AGP enhances the ability of the amphipathic mediator to induce a thermoregulatory response, as does its binding to albumin (22, 49). To answer this question for the case of the pyrogenic activity of

the AGP-bound form of PAF, a highly potent mediator of LPS action, was the second aim of the present study.

We have found that the intravenous [PAF-AGP] complex is much more pyrogenic than free PAF. The [PAF-AGP] complex, but not the free form, produced a marked fever with a very short latency. Similarly to fevers induced at thermoneutrality by LPS (66), PGE_2 (5, 64), or a [PAF-albumin] complex (22), the fever evoked by the [PAF-AGP] complex was brought about, at least partially, by tail skin vasoconstriction (as shown by the associated decrease in the HLI). This is the first demonstration of the ability of a ligand to exhibit a pyrogenic activity when bound to AGP. It is also the first demonstration of an enhanced pyrogenic activity of a ligand in the AGP-bound form. We conclude that circulating AGP may participate in immune-to-brain signaling by transporting PAF and possibly other amphipathic inflammatory mediators in genetic analbuminemia.

Perspectives

The proposed role of AGP can be extended to other cases of hypoalbuminemia. For example, systemic inflammation and sepsis are accompanied by a moderate (up to 2-fold) decrease in the plasma level of albumin (21, 58) and strong (~15-fold) increase in the concentration of AGP (43), which unavoidably results in redistribution of ligands among carrier proteins (21, 71). Under such conditions, the importance of AGP-mediated transport can be expected to increase drastically.

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GRANTS

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