Posthemorrhagic antipyresis: what stage of fever genesis is affected?

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Romanovsky, Andrej A., and Yelena K. Karman. Post-hemorrhagic antipyresis: what stage of fever genesis is affected? J. Appl. Physiol. 83(2): 359–365, 1997.—It has been shown that hemorrhage leads to a decreased thermal responsiveness to lipopolysaccharide (LPS). The aim of this study was to clarify what stage of fever genesis [production of endogenous pyrogens such as interleukin-1 (IL-1), increase of the prostaglandin E2 (PGE2) concentration in brain tissue, activation of cold-defense effectors] is deficient in posthemorrhagic antipyresis. In adult rabbits, we evaluated the effect of acute hemorrhage (15 ml/kg) on the rectal temperature (Tre) responses to LPS from Salmonella typhi (200 ng/kg iv), ethanol-purified preparation of homologous IL-1 (1 ml from 3.5 × 107 cells, 1.5 ml/kg iv), and PGE2 (1 µg intracisternal injection). The effect of hemorrhage on T

Despite the fact that both fever and hemorrhage are common signs of disease, their potential interactions are still poorly understood. One aspect of the problem, i.e., how the pathophysiology of hemorrhage changes under fever conditions, will not be addressed in this paper. We studied the other aspect, i.e., how hemorrhage modifies the thermoregulatory response to systemic inflammation.

There is anecdotal evidence that physicians have been familiar with an antipyretic effect of bloodletting for a long time. In the early 1800s, the English surgeon Lionel Wafer wrote: "I bound up her arm ... and with my lancet breathed a vein ... and I drew off about 12 ounces ... and desired she might rest till the next day; by which means the fever abated and she had not another fit" (cited from Ref. 10). Yet, experimentally, the posthemorrhagic attenuation of the febrile response was rediscovered only recently. In 1980, Kasting and colleagues (8) observed such an attenuation in acutely hemorrhaged sheep administered an exogenous pyrogen. This first communication was followed by a detailed report (9) and, in a few years, by another study by Kasting (7) showing that the hemorrhage-induced attenuation of lipopolysaccharide (LPS)-induced fever similarly occurs in the rat. A decade later, we reproduced the same phenomenon in yet another species, the rabbit, as described in a preliminary report of the present work (17). We termed the observed phenomenon posthemorrhagic antipyresis.

The mechanisms of the posthemorrhagic antipyresis remain speculative. Although it is conjectured that it occurs because of a release of arginine vasopressin (AVP) in the brain and an action of the peptide on central febrile pathways (7, 9), the proposed mechanism has never been elucidated in direct experiments. Nor has any alternative mechanism [e.g., blockade of the synthesis and/or release of pyrogenic cytokines such as interleukin (IL)-1, IL-6, tumor necrosis factor (TNF), and interferons] been seriously considered or convincingly ruled out. The aim of the present study is to clarify what stage of fever genesis is affected by hemorrhage. In particular, we tested whether posthemorrhagic antipyresis could be due to any of the following factors: 1) incapability of the hemorrhaged organism to achieve an effective blood concentration of IL-1, 2) inability to secure a sufficient rise in the cerebrospinal fluid concentration of prostaglandin E2 (PGE2), and 3) obvious or hidden functional incompetence of thermoregulatory effector mechanisms.

METHODS

Animals

Sixty-two male Chinchilla rabbits weighing ~3 kg were used in this study. They were housed in individual cages, under the conditions of 12:12-h light-dark cycle (lights on from 8:00 AM), at an ambient temperature (T

Experimental Protocols

Six experiments were conducted. Five of them (experiments 1–5) were designed to compare thermal responses to various stimuli between the two groups of rabbits, i.e., hemorrhaged (immediately before the stimulus application)
and intact. In experiment 1, the stimulus was an intravenous injection of LPS; in experiment 2, the stimulus was represented by an intravenous administration of homologous IL-1; in experiment 3, the thermal response was induced by an intracisternal injection of PGE_2; in experiment 4, no stimulus (other than an exposure to the experimental conditions) was applied; and in experiment 5, the thermal response to a cold exposure was studied. For experiments 1–4, no animal instrumentation or restraint was required, and the rabbits were kept in open plastic boxes (approximate dimensions are 100 cm (length) × 50 cm (width) × 30 cm (height)). Their rectal temperature (T_r) was taken with an electronic thermometer at 10- to 30-min intervals. T_a was maintained at 23°C. In experiment 5, the rabbits were instrumented with thermo-couple probes and lightly restrained with homemade conventional-type stocks that prevented the animals from turning around and restricted their back-and-forth movements but did not affect their normal posture. T_r was continuously monitored. Immediately after blood was withdrawn, these rabbits (in their stocks) were transferred into a temperature-controlled chamber (model Prima, Levin Workshop, Minsk, Belarus), and its air temperature was decreased by ramp cooling from 23 to 7°C; to maximize the effect of cooling, no dehumidification was used, and the air inside the chamber was vigorously mixed with a fan. A separate experiment (experiment 6) was conducted to assess the effect of acute hemorrhage on the plasma AVP level and thus to verify the effectiveness of this degree of blood loss to induce hormonal changes. For this experiment, the same protocol as in experiment 4 was followed, but no thermometry was performed. Instead, 2-ml samples of blood (to be analyzed for AVP later) were taken from a marginal ear vein, one 30 min before and the other 10 min after the hemorrhage.

Hemorrhage

The caudal margin of the right ear pinna was gently compressed over the most proximal portion of the marginal vein, and a 2- to 3-mm incision of skin together with a wall of the underlying vein was made 1.5–2.5 cm distal to the place of compression. Before the procedure, the skin was shaved, disinfected with ethyl alcohol, and treated with xylene to induce local vasodilation. Blood (15 ml/kg; equivalent to 20% of the blood volume) was then gathered in a collection vessel, and a miniature hemostatic clip was placed over the incision thereafter. During the procedure, the animal remained in its plastic box and was gently restrained by hand to prevent accidental movements. Because rabbits tolerate this short-lasting (4- to 7-min) manipulation without any apparent autonomic or behavioral reactions, no anesthesia was used. The 15 ml/kg amount of blood withdrawn was chosen because such a hemorrhage does not cause macrohemodynamic alterations (for an extensive review, see Ref. 23) yet results in obvious changes of the hormonal status (for details, see DISCUSSION), including a dramatic increase in AVP secretion (9).

Drugs and Drug Administration

Drugs injected intravenously. A commercially available LPS from Salmonella typhi (series 306–3; N. F. Gamaleya Institute of Epidemiology and Microbiology, Moscow, Russia) and rabbit IL-1 (ethanol purified; 1 ml from 3.5 × 10^7 cells of peritoneal exudate; Institute of Experimental Medicine, St. Petersburg, Russia) were injected intravenously (in a marginal ear vein) in a dose of 200 ng/kg and 1.5 ml/kg, respectively. The IL-1 preparation used was endotoxin-free and possessed high thymocyte-proliferating and pyrogenic activities. LPS was suspended in saline and stored at 4°C. IL-1 was aliquoted, frozen, and kept at −20°C; no sample of IL-1 was frozen and thawed more than twice.

Drugs injected intracisternally. PGE_2 (Sigma Chemical, St. Louis, MO) was dissolved in a 5% ethanol solution, aliquoted, frozen, and stored at −20°C; each aliquot was thawed only once. The drug was administered intracisternally, at a dose of 1 µg, in a 100-µl volume. For an injection, the rabbit was taken out of the box and, with the help of a specially designed stock, fixed lying on its belly, with the maximal possible flexion in the atlanto-occipital joint. The skin of the occiput and nape (that had been shaved previously) was disinfected. Soft tissues over and caudal to the external occipital protuberance were infiltrated with a local anesthetic (Novocain, 1%, 0.5 ml). Thereafter, the atlanto-occipital membrane (together with its overlaying tissues) was punctured with the help of a short-bevel 18-gauge injection needle (with a mandrel inserted in the needle). After the mandrel was removed and the correct position of the needle verified by the appearance of a drop of the cerebrospinal fluid, the needle was connected to a microsyringe and an injection into the cisterna magna was made. Thereafter, the rabbit was released from the stock and returned to its box. The experimenters underwent special training to perform the intracisternal puncture; the entire procedure never lasted >2 min.

Thermometry

In experiments 1–4, T_r was measured with an electronic thermometer (Institute of Experimental Medicine); the probe length was 5 cm. In experiment 5, T_r was measured with a copper-constantan thermocouple inserted 7 cm beyond the anus. The reference junction of the thermocouple was kept at 37.0°C; the signal from a thermocouple was fed to a direct-current amplifier (model F116/2, KINAP, St. Petersburg, Russia) and sent to a recorder (model KSP-4; Institute of Experimental Medicine).

AVP Assay

Blood (2 ml) was collected into chilled plastic tubes each containing 200 µl of saturated Trilon B solution and immediately centrifuged (5,000 m×g; 10 min). Plasma was transferred to new tubes, frozen, and stored at −20°C until the analysis. AVP concentration (±1 pg/ml) was determined with the help of a radioimmunoassay kit (antidiuretic hormone; Bühmann Laboratories, Basel, Switzerland); for plasma samples, the analysis was preceded by ethanol extraction of protein according to the kit manual.

Data Analysis

Data (means ± SE) are presented in the form of either the absolute value or the deviation (Δ) of a parameter from its preinjection level (averaged over 60 min). To compare two T_r curves, we integrated the ΔT_r functions over the entire length of the experiment and treated the obtained integrals by using the unpaired Student’s t-test. To compare the blood levels of AVP before and after hemorrhage (experiment 6), the paired Student’s t-test was used.

RESULTS

In all the experiments, the initial values of T_r were similar, with the mean for the whole study being 39.2 ± 0.1°C. In experiment 1, control animals responded to LPS with a typical biphasic fever; this response was greatly attenuated (P < 0.027) and became monophasic under the conditions of hemorrhage (Fig. 1). At the time
of the second phase (4 h postinjection), $\Delta T_{re}$ was only 0.4 ± 0.1°C compared with 1.2 ± 0.2°C in the nonhemorrhaged controls. In experiment 2, IL-1 caused a monophasic fever, which did not change its shape but was substantially (by ~0.5°C) and significantly ($P < 0.005$) attenuated in hemorrhaged animals throughout the period of observation (Fig. 2). In experiment 3, PGE$_2$ caused a typical (rapid and short-lasting) rise in $T_{re}$ of the controls; in the hemorrhaged rabbits, this thermal response was both delayed and attenuated ($P < 0.048$; Fig. 3). The attenuation persisted over the first 2 h postinjection, reaching its maximum at 45 min when $\Delta T_{re}$ was 0.9 ± 0.1°C in the controls but only 0.4 ± 0.1°C in the hemorrhaged rabbits. By itself, the hemorrhage neither induced any hypothermia under thermoneutral conditions (experiment 4 and Fig. 4) nor influenced the rabbits’ thermal responses to an external cooling (experiment 5 and Fig. 5). Although the chosen volume of blood loss (15 ml/kg) was subthreshold for affecting the thermal balance in afebrile animals, it was sufficient to cause a >14-fold rise (from 5 ± 3 to 71 ± 15 pg/ml; $P < 0.003$) in the plasma AVP concentration in experiment 6 (Fig. 6).

**DISCUSSION**

Mechanisms of Posthemorrhagic Antipyresis

The results of experiment 1 showed that the febrile response to LPS, which is known to be attenuated by hemorrhage in sheep (9), rats (7), and presumably humans (10), is similarly affected in hemorrhaged...
rabbits. What stage of fever pathogenesis is influenced by hemorrhage? In this study, we have examined several possibilities.

Posthemorrhagic antipyresis: is it due to a decreased production of endogenous pyrogens? Because hemorrhage results in the mechanical removal of a substantial number of blood cells (including leukocytes, which are producers of cytokines), it might be suggested that LPS-induced cytokine synthesis is abated in hemorrhaged animals. Yet, depletion of blood phagocytes is unlikely, by itself, to have any physiologically meaningful effect on the production of pyrogenic cytokines: first, it is well established that leukocytes from even minute quantities of blood are capable of producing a sufficient amount of endogenous pyrogens to cause substantial fevers (6); and, second, blood removal does not affect (at least directly) the number of residual, tissue producers of cytokines (Kupffer cells, microglia, etc.).

Hemorrhage is also known to drastically alter the hormonal pattern of blood by activating the hypothalamic-pituitary-adrenal, renin-angiotensin-aldosterone, and several other components of the endocrine system (for an example of hormonal changes induced by a 15 ml/kg hemorrhage in the sheep, see Ref. 4). The list of affected hormones includes catecholamines, adrenocorticotropic hormone (ACTH), glucocorticoids, angiotensin II, enkephalins, β-endorphin, insulin, vasoactive intestinal peptide, and others. In the present work, the effectiveness of a 15 ml/kg hemorrhage to induce hormonal changes was verified by a >14-fold increase in the plasma AVP concentration (experiment 6), which is consistent with the literature (9). The hemorrhage-associated shifts in the blood hormonal pattern can readily affect various immunocyte functions, including the synthesis and release of pyrogenic cytokines (14). Yet, because of the multiplicity of the endocrine-immune interactions, it is difficult to predict the overall effect on cytokine production. Attempts to address this issue in direct experiments led to contradictory results. Thus there are some data showing that, in the rat, a 30% hemorrhage attenuated the LPS-induced rise in the plasma IL-6 concentration 24 h postbleeding (12). In accord with this, LPS-induced surges in blood IL-6 and TNF-α were shown to be blocked over the first several hours postbleeding in the rat model of a 20% hemorrhage (D. Soszynski, personal communication). Yet, several studies have shown that the processes of cytokine gene transcription in a wide spectrum of peripheral tissues, the ability of various cell types to produce pyrogenic cytokines, and the blood level of several endogenous pyrogens are all increased during the first several hours posthemorrhage, sometimes for as long as 24 h (5, 12, 21). Much work is still needed to convincingly answer the question of whether hemorrhage attenuates the synthesis and/or release of pyrogenic cytokines.

One of the questions we tried to answer in the present study is whether the inability of the organism to synthesize sufficient amounts of pyrogenic cytokines (or, in particular, IL-1) could be the major reason for the development of the posthemorrhagic antipyresis. If it were, fevers induced by exogenous pyrogens (those that require de novo synthesis and release of pyrogenic cytokines) could be attenuated, but febrile responses to endogenous pyrogens (those that do not require cytokine synthesis) should not be compromised. This was not the case in our experiment 2: the hemorrhaged rabbits responded to IL-1 with a substantially attenuated fever compared with the controls. We conclude, therefore, that bypassing the stage of cytokine production and release (by administration of already-synthesized IL-1 directly into the blood) does not obviate posthemorrhagic antipyresis. In other words, the mechanisms of the phenomenon would not seem to involve (at least as the major and/or only contributor) the blockade of the production of IL-1.
Can posthemorrhagic antipyresis be due to an insufficient rise in brain prostaglandins? If PGE₂ plays a role in fever genesis, hemorrhage could be thought to affect the febrile response by inhibiting the rise in the brain PGE₂ concentration. It could then be expected that fevers induced by either exogenous or endogenous pyrogens might be attenuated by hemorrhage but that the thermal response to exogenous PGE₂ delivered into the brain directly should not be affected. This was not the case in experiment 3, in which the PGE₂-induced Tₑ rise was substantially attenuated in hemorrhaged animals compared with the nonhemorrhaged controls. This suggests that the posthemorrhagic antipyresis affects a stage of fever downstream from a rise in PGE₂ concentration in the brain tissue.

Posthemorrhagic antipyresis: a weakness of thermoeffectors? In the present study, hemorrhage attenuated fevers induced by LPS and IL-1 as well as hyperthermia induced by PGE₂. Could it be possible that, in all three cases, the hemorrhaged animals did not develop the same Tₑ rise as did the naive controls because their thermoeffectors (heat production and/or heat conservation) were affected? Hemorrhage, if severe enough, could result in hemodynamic alterations (shock, in an extreme case) and hypoxia; both hypoxia and compromised hemodynamics might affect various peripheral functions. The volume of blood loss chosen for our experiments (20%) was sufficient for inducing profound hormonal changes, as judged from a dramatic rise in the blood AVP (experiment 6). Yet, such a volume is known to be subthreshold for the development of any macrohemodynamic alterations: an acute loss of 25% of the blood volume is usually rapidly compensated for as a result of hemodilution, flow redistribution, and other factors; clinical symptoms of blood loss occur only if it reaches 25% or more of the initial volume; systemic arterial pressure usually does not decrease after hemorrhage if the blood loss is below 25–30%; and tachycardia does not usually occur in surgical (heart) patients even in cases of 25–30% blood loss (23).

We addressed the issue of possible posthemorrhagic thermoeffector deficiency in experiments 4 and 5. The results indicate that, after a 15 ml/kg (20%) hemorrhage, afebrile (not injected with any pyrogen) rabbits are capable of maintaining their Tₑ at the same level as did the controls in both thermoneutral (experiment 4) and cold (experiment 5) environments. In the latter case, cold exposure should be regarded as severe for the rabbits maintained at (acclimated to) 23°C: their Tₑ decreased by >1°C, and the effective Tₐ was probably much lower than 7°C because of high air humidity and velocity (see Experimental Protocols). Our observation of cold defense remaining competent in hemorrhaged rabbits is in agreement with the literature: the same degree of blood loss (20%) resulted in no apparent thermoregulatory deficiency either in rats exposed to a slightly cool environment, i.e., room temperature (Ref. 7; D. Soszynski, personal communication), or in sheep exposed to a moderate (4°C) cold (9). Because effectors of cold defense (as tested in 3 species with a minimal-to-severe cold exposure) seem to work normally in animals with a 20% hemorrhage, the posthemorrhagic antipyresis is unlikely to represent a result of thermoeffector insufficiency.

What stage of the fever genesis is affected by hemorrhage? The major question we tried to answer in the present work is not a trivial one. Part of the problem is that the current ideas of the pathomechanism of the febrile response are not very clear (for review on controversial issues in the current concept of fever genesis, see Refs. 3, 24). Are pyrogenic cytokines the first (earliest) endogenously released mediators of fever (as it is generally believed), or, alternatively, may anaphylatoxic complement components, C₃a and C₅a, play this role? How does the pyrogenic signal reach the brain: is it through a humoral pathway only (as it is commonly thought) or via a neural path as well? Are prostaglandins involved in the pathogenesis of fever at all, and, if they are, are they blood borne or of local (cerebral) origin? Without the answers to such questions, it seems difficult to specify, with a certain degree of confidence, the stage of fever affected by hemorrhage (or, to this end, any another disturbance). Yet, the present work allows us to exclude several potentially possible mechanisms.

The present experiments show that hemorrhage attenuates not only LPS-induced fever but also the febrile response to an endogenous pyrogen (IL-1) and the PGE₂-induced hyperthermia. This means that neither the synthesis of pyrogenic cytokines nor the processes leading to the rise in the brain level of PGE₂ are likely to constitute the only and/or main reason of the posthemorrhagic antipyresis. On the other hand, hemorrhaged animals seem to be capable of mounting sufficient cold-defense responses; the thermoeffector insufficiency could, therefore, also be excluded as the important underlying mechanism. This would mean that hemorrhage affects fever genesis at a stage after the release of IL-1 into the blood and the rise of the intrabrain PGE₂ concentration but preceding the activation of heat-production and/or heat-conservation effectors.

We speculate that this stage might be the action of PGE₂ (and other propyretic mediators) on neurons (perhaps warm sensitive) "wired" to thermoeffectors. In nonhemorrhaged animals, fever mediators modify the activity of these neurons and thus trigger the appropriate thermoeffector responses. Under the conditions of hemorrhage, a centrally released substance or, even more likely, substances (endogenous antipyretics) modify the chemoresponsiveness of these neurons; this is why the thermal responses to LPS, IL-1, and PGE₂ were all blocked in the hemorrhaged rabbits. Yet endogenous antipyretics do not affect the responsiveness of the same neurons to other (nonfebrile) stimuli such as temperature per se; this is why the thermal responses to cold are apparently not affected by hemorrhage. This hypothesis does not contradict the original explanation by Kasting and colleagues (7, 9), who speculated that the posthemorrhagic antipyresis occurs as a result of endogenous AVP released in the brain and affecting the central febrile pathways. We might add, however, that
not only AVP but also other neuropeptides with putative antipyretic activity (ACTH, angiotensin II, α-melanotropin, etc.) are likely to contribute to the development of the posthemorrhagic antipyresis. The detailed characterization of the mechanisms of this phenomenon, including localizing of the responsible neurons in the brain and drawing their functional portraits, awaits further investigation.

Posthemorrhagic Antipyresis: Biological and Clinical Significance

Under natural conditions, hemorrhage is usually associated with trauma, the consequent disintegration of the barrier between the internal and external environments, and, potentially, infection. It is dear that hemorrhage, if severe enough, can readily impair various functions of the organism, including its defense against infection (5). Yet, the repeated occurrence of the infection and the vital importance of its outcome could have resulted in the evolutionary development of some adaptive interactions between hemorrhage, on the one hand, and various mechanisms and manifestations of the infectious process, on the other hand. The relationship between fever and hemorrhage described in this paper could represent an example of such adaptive interactions. If so, what is the biological significance of the phenomenon of posthemorrhagic antipyresis? Let us try to answer this question from the position of the dual thermoregulatory strategy of adaptation to systemic inflammation (for details, see Refs. 18, 20).

We have hypothesized that fever and hypothermia represent two adaptive responses, each developed under certain conditions and each beneficial under these conditions. The antimicrobial and immunostimulating benefits of a high body temperature (for review, see Ref. 11) could be easily offset by its high energy cost; fever, therefore, is protective only when there is no immediate threat of a substantial energy deficit. Hypothermia, on the other hand, constitutes a response aimed at energy conservation and, as such, is beneficial exactly under the conditions of a substantial energy deficit: whether it is septic (1), endotoxin (19) or hemorrhagic (2, 15) shock, hypothermia seems to possess a recuperative value. Whenever the conditions are unfavorable (stress, cold exposure, hypoxemic hypoxia, etc.) or the organism is particularly vulnerable (pregnancy, neonatal period, hemorrhage, etc.) and the threat of energy deficiency becomes real, a decrease in body temperature (hypothermia and antipyresis) becomes beneficial. It is not by chance, therefore, that antipyresis can be induced by such heterogeneous stimuli and conditions as hemorrhage (7, 9), malnutrition (22), a severe osmotic load (7, 16), and restraint (13), as well as many others. An understanding of the adaptive value of such a phenomenon could change our approach to several clinical situations, including those that involve a substantial blood loss.

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