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Circadian Variation in Circulating Pyrogen: Possible Role in Resistance to Infection (41649)

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Abstract. In the rat, body temperature (bt) is highest, and plasma iron (Fe) and zinc (Zn) concentrations are lowest at night while the rat is most active; the inverse is true during the day. Based on data implicating endogenous pyrogen (EP) as a mediator of the rise in body temperature and fall in plasma trace metal levels during infection we hypothesized that the circadian rise in body temperature and fall in plasma Fe and Zn levels may be attributed to a cyclic release of EP. To test this hypothesis: (1) Rats were injected ip with an antipyretic dose of sodium salicylate (300 mg/kg). The result was a reduction ($P < 0.05$) in bt at night. (2) Rats were injected during the day with 1 ml each of plasma collected from rats during the night. As a control, rat plasma collected during the day was injected at this same time point. A rise ($P < 0.05$) in bt was observed only in animals who had received plasma collected at night. These results support the hypothesis that a pyrogen, perhaps EP, is present in the plasma of rats at night. The release of EP during periods of greatest activity may have an adaptive role since rats are more likely to come into contact with pathogens during these times. If EP were released during periods of activity, the likelihood of severe infection occurring would be diminished. To test this hypothesis, two groups of rats were injected with *Salmonella typhimurium,* one group at midnight (A) and one group at noon (B). The mortality rate was 25% in group A and 60% in group B ($P < 0.025$). These data support the hypothesis that the immune/host defense of rats to *S. typhimurium* is more effective at night, possibly due to an increased level of circulating pyrogen.

Circadian variation of plasma concentrations of iron (1–3) and zinc (4, 5), and body temperature (2, 6) occurs in many animals. A preliminary study in our laboratory has shown that in the rat, the circadian changes in iron and zinc are inversely correlated to changes in body temperature. A similar inverse relationship between a rise in body temperature and fall in plasma levels of iron and zinc is generally observed during infection. Some of the "acute phase" responses observed during infection, including fever and hypoferremia–hypozincemia, are thought to be mediated by a small-molecular weight protein, endogenous pyrogen (EP) (7–10). Many of the effects of EP are thought to be beneficial to the infected host. For example, there are numerous studies indicating that moderate fevers are beneficial (11, 12). A reduction in plasma iron has been shown to decrease the growth rate of several species of bacteria (13–16), thereby benefiting the infected host.

We hypothesized that the circadian variations in temperature and trace metals could, in part, be the result of a circadian release of EP. To test our first hypothesis we (i) injected rats with an antipyretic dose of sodium salicylate to determine whether we could attenuate the circadian rise in body temperature, i.e., "block the fever," and (ii) collected blood from rats during the night and day to assay the plasma for the presence of pyrogens using a rat bioassay recently developed in our laboratory (17, 18). If our hypothesis that the circulating level of EP is highest at night is supported by our data, then we predict that the resistence of rats to infection with a pathogenic microorganism, *Salmonella typhimurium,* will also be greatest at night. To test whether survival is enhanced in rats infected at night, we injected two groups of rats with equal doses of *S. typhimurium* at noon and midnight and compared mortality rate.

Materials and Methods. *Experiment 1: Circadian variation in body temperature and plasma trace metal levels.* Male Sprague–Dawley rats, weighing 170–230 g, were housed in individual cages and maintained on a 12-hr light/dark photoperiod in a temperature-controlled chamber at 26 ± 1°C. The body temperatures of the rats were measured using a flexible 2-mm-diameter thermistor probe inserted approximately 6 cm into the rectum. The temperature data were read and recorded

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off the display of a digital temperature monitor (±0.1°C).

Rectal temperatures and plasma iron and zinc concentrations were determined every 4 hr for 24 hr. Blood was collected for trace-metal analysis by decapitation following the temperature measurements.

Whole blood was collected into heparinized polystyrene tubes, centrifuged at 2500*g* for 10 min, and the plasma drawn off. For the analysis of plasma concentration of zinc, equal volumes of plasma and 1.22 *M* trichloroacetic acid were mixed and left to stand for 30 min. For determination of plasma iron concentration, equal parts of 1.22 *M* trichloroacetic acid and plasma were mixed and subsequently heated at 90°C for 15 min to denature proteins. Both sets of samples were centrifuged at 2500*g* for 10 min. The supernatant was drawn off and saved for testing. The zinc and iron concentrations were measured using the method of atomic absorption spectrophotometry (model Varian 375). Three to four absorbance values were obtained per sample over a 3-sec integration period.

Experiment 2: Effect of sodium salicylate (SS) on the circadian rise in body temperature. Ambient temperature was maintained at 27 ± 1°C, well within the thermoneutral zone of the rat, to ensure that heat/cold stress would not be a factor affecting the response to SS. Rats were housed two to a cage to alleviate isolation stress and to allow for behavioral thermoregulation. One of the pair received SS while the other received saline.

Experimental animals (N = 11) were injected intraperitoneally (ip) with 0.2 ml of 300 mg/kg SS; control animals (N = 11) were injected ip with 0.2 ml of 0.9% sodium chloride. Rectal temperatures were measured just prior to the injection at 8:00 p.m. The temperatures of all animals were monitored at 1-hr intervals until midnight.

Another experimental group of animals (N = 14) was injected with the same dose of SS at 11:00 a.m. Control animals (N = 10) received 0.9% sodium chloride. Rectal temperatures were monitored at 1-hr intervals until 3:00 p.m.

Experiment 3: Effects of injections of rat plasma on body temperature. Rats were housed and maintained the same as in Experiment 2. Whole blood was collected by decapitation into heparinized polystyrene tubes from seven rats at midnight and from six rats at 11:00 a.m. After centrifugation for 10 min at 2500*g*, the plasma was drawn off and pooled with plasma collected at the same time point. Plasma samples were stored at −20°C for no longer than 72 hr before being bioassayed for pyrogen.

Rats were injected ip with 1 ml of the plasma collected at midnight (N = 13) or 1 ml of the plasma collected at 11:00 a.m. (N = 11). The rectal temperatures of rats used in our bioassay were measured prior to injection at 9:30 a.m. We selected 9:30 a.m. to inject rat plasma since body temperature tends to be fairly stable from this time until sometime after 4:00 p.m. This time is also a low point for body temperature. Rectal temperatures were measured 90 min following injection of plasma; from previous work in our laboratory, it was determined that maximum response to plasma EP from humans is elicited 90 min after injection (17, 18). In addition, we have been able to repeatedly produce an endogenous pyrogenlike substance from stimulated rat mononuclear cells that induces fevers, as determined at 90 min, when injected into rats (J. Cannon and D. McCarthy, unpublished observations).

Experiment 4. Effect of time of day on survival of Salmonella-infected rats. Salmonella typhimurium, stored in glycerol at −20°C, was cultured on blood agar plates and incubated at 37°C for 14 hr. A concentration of approximately 8.0×10^8 bacteria/ml was prepared and 0.2 ml injected intravenously (iv) into 10 rats at noon. The same preparation of bacteria was stored at 4°C to be injected into 10 rats at 2400 hr (midnight). Thirty minutes prior to injection at this time, the bacteria were placed in an incubator (37°C) to restore their normal metabolic rate. The same experiment was repeated in the reversed order, i.e., the bacteria were prepared just prior to injection at midnight and refrigerated for use at noon. The procedure was reversed to eliminate any discrepancy due to possible "die-off" of bacteria colonies during their storage at 4°C for 12 hr prior to injection. Serial dilutions and subsequent plate counts at the two different times of injection were determined and these data confirmed that the numbers of bacteria the two groups received were similar

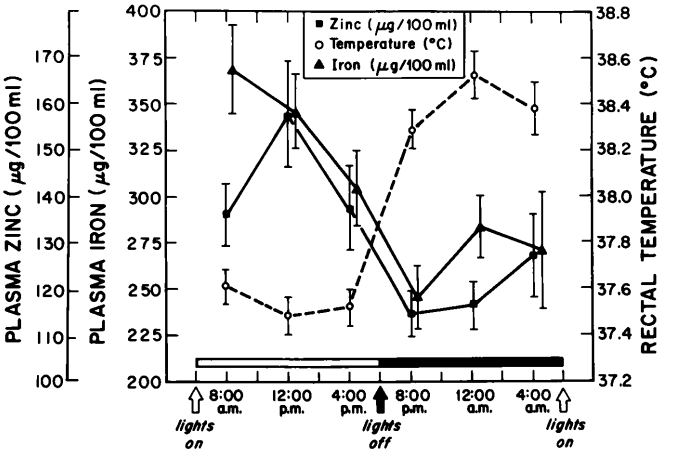

FIG. 1. The normal circadian variation in rectal temperature (°C) and plasma concentrations of iron and zinc (μg/100 ml) in male Sprague–Dawley rats (N = 8 for each time point). Values represent mean ± 1 SEM.

(average dose of bacteria injected at noon = 7.2×10^8; at midnight = 7.9×10^8). The number of surviving animals were counted every 12 hr for the duration of the experiment. The study was terminated 72 hr after the injection of *S. typhimurium*.

Results. Figure 1 illustrates the inverse relationship between circadian changes in body temperature and the plasma trace metals, iron and zinc. Mean body temperature was found to be highest at midnight and lowest at noon. Plasma concentrations of iron and zinc were lowest at 8:00 p.m. and highest at 8:00 a.m. and noon, respectively.

The effect of sodium salicylate (300 mg/kg) on the normal body temperature of rats at night is shown in Fig. 2a. This drug, administered at a dose known to be antipyretic in EP-induced fevers, effectively depressed the normally high body temperature of the rat observed at night throughout the 4-hr period following injection ($P < 0.01$). A significant difference in body temperature between SS-injected rats and saline controls was observed

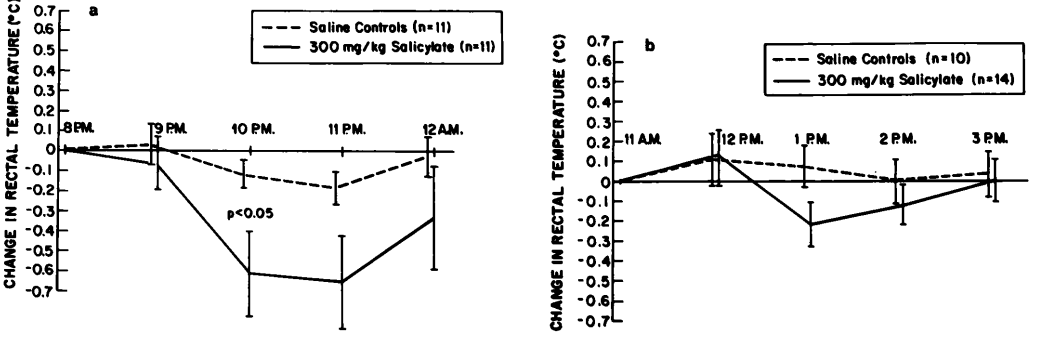

FIG. 2. (a) Change in rectal temperature of male Sprague–Dawley rats injected with 300 mg/kg sodium salicylate (N = 11) or saline (N = 11) at 8:00 p.m. A statistically significant difference was found in rectal temperature both at the specific time-point of 10:00 p.m. and for the average change in rectal temperature for the entire 4-hr period. Values represent mean ± 1 SEM. (b) Change in rectal temperature of male Sprague–Dawley rats injected with 300 mg/kg sodium salicylate (N = 14) or saline (N = 10) at 11:00 p.m. No statistical significance between the two groups was found at any point. Values represent mean ± 1 SEM.

at 10:00 p.m., 2 hr after injection ($P < 0.05$) and for the average change in rectal temperature for the entire 4-hour period. There was no significant difference between the change in body temperatures of rats injected with 300 mg/kg of SS or saline during the day (after injection at 11:00 a.m.) (See Fig. 2b).

The response of rats injected at 9:30 a.m. with plasma collected at midnight and 11:00 a.m. is shown in Fig. 3. The body temperatures of the experimental group injected with the midnight plasma was an average of 0.33°C greater than the temperatures of the control group injected with 11:00 a.m. plasma ($P < 0.05$).

Figure 4 shows the results of the survival study conducted beginning at midnight and noon. The difference in mortality at 72 h between the group infected at midnight and the group infected at noon was significantly different using a chi-square test ($P < 0.025$).

Discussion. These data support the hypothesis that at least part of the rise in body temperature observed at night is in effect a "fever" (i.e., the central thermostat is reset at a higher level) and, as a result, the elevated body temperature can be reduced with the antipyretic drug, sodium salicylate (19). Further, since sodium salicylate is known to exert its effects through inhibition of prostaglandin synthetase (20), we conclude that prostaglandins are likely to be involved in the circadian rise in body temperature of the rat at night.

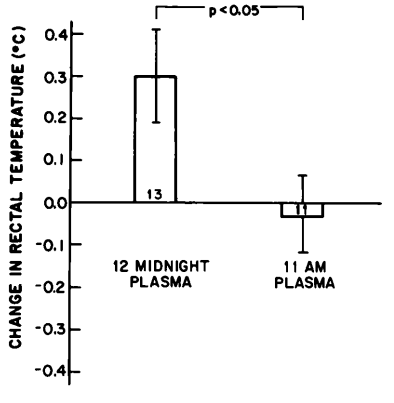

FIG. 3. Change in rectal temperature of rats injected with 1 ml of plasma collected from rats at midnight ($N = 13$) or 11:00 a.m. ($N = 11$). The changes in temperature were determined at 1100 hr, 90 min after injection at 9:30 a.m. Values represent mean ± 1 SEM.

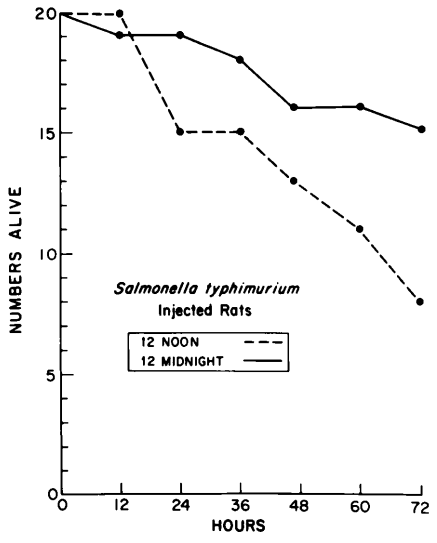

FIG. 4. Survival of male Sprague–Dawley rats infected with *Salmonella typhimurium* at noon ($N = 20$) and midnight ($N = 20$). Mortality was determined every 12 hr up to 72 hr after injection.

Further support for the hypothesis that there is a circadian variation in circulating pyrogens is based on results obtained from bioassaying the plasma of rats. These results indicate that there is a pyrogenic factor present in the plasma of rats at midnight, when body temperature is high; this factor is either inactive or absent from the plasma of rats at 11:00 a.m. when body temperature is low. We cannot conclude, from these data, whether this pyrogen is actually EP. Experiments are planned to characterize further this pyrogen.

We speculate that the decreased mortality rate in rats injected with *S. typhimurium* at midnight compared to rats injected at noon may be related to an increased level of circulating endogenous pyrogen. An EP-like substance is found circulating in the plasma of human subjects following strenuous exercise (17, 18) indicating that plasma concentrations of EP may rise even without the occurrence of infection. There are considerable data implicating EP as the initiator of many of the acute phase responses to infection (9, 21) and therefore an elevated level of EP may have protective value. One study has reported that injecting EP into rats is indeed protective (22). We speculate that rats are more likely to come into contact with pathogens when

foraging for food, mating, defending territories, etc. If EP were released during these periods of activity, i.e., at night, the likelihood of severe infection occurring may be diminished. The enhanced resistance to infection at night may be related to an increased level of circulating EP or alternatively, to some undetermined circadian variation.

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