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CIRCADIAN VARIATION IN HOST DEFENSE(U) MICHIGAN UNIV
ANN ARBOR DEPT OF PHYSTIOLOGY M J KLUGER 18 NOV 85
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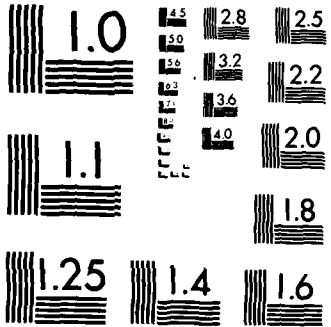
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Figures 2 and 3). Administration of this same dose of sodium salicylate to rats during the morning hours had no effect on body temperature or on activity. Since antipyretic drugs are thought to act by returning an elevated thermoregulatory "set-point" to normal, these data suggest to us that the rise in temperature observed at night in rats is a true fever, perhaps induced by interleukin-1 (IL-1). To eliminate any systemic effects of sodium salicylate, we are currently testing the hypothesis that administration of microgram amounts of sodium salicylate and indomethacin directly into the lateral cerebral ventricles will attenuate the nocturnal rise in body temperature. We are hoping to obtain, within the next few months, antibodies to IL-1 from Dr. John Schmidt of Merck Laboratories. These antibodies will be injected into the central nervous system to determine whether antibodies to IL-1 will also attenuate circadian changes in body temperature.

We also plan to investigate whether a circadian variation in IL-1 exists in human beings. Although claims are made that antipyretic drugs have no effect on the body temperature of afebrile human subjects, to our knowledge, this has never been carefully tested. Permission has been obtained from our Clinical Research Center to study circadian variations in body temperature, trace metals, white blood cell concentrations, and plasma IL-1 concentrations in 10 human subjects. We will determine whether the afternoon rise in body temperature in human subjects can be attenuated by administration of aspirin. Each subject will have his/her body temperature recorded at 10 minute intervals. On day 1 of each experiment, the subject will be given placebos at 4 hour intervals. On day 2 of each experiment, the subject will either be given placebos or aspirin at 4 hour intervals. Blood samples will be taken at 4 hour intervals. The effects of aspirin on body temperature, trace metals, and white cell count will be determined. Plasma concentrations of IL-1 will be determined using the in vitro thymocyte proliferation assay.

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Objectives

To determine whether interleukin-1 (IL-1), the protein responsible for many of the nonspecific acute phase responses to infection (e.g. fever, leukocytosis, changes in plasma trace metals, etc.), is released on a circadian basis.

1. Accomplishments During the First Year of Support:

Progress during the first year of support has been slower than anticipated largely because about six months elapsed before we could get our temperature and activity telemetry system running adequately to begin collecting data. Our data collection system, the Dataquest III from Mini-Mitter, was the first telemetry system designed by Mini-Mitter for use with an IBM PC. While the use of an IBM dependent system allows for better data acquisition and analysis, the new system had several technical problems, some of which have only recently been corrected.

We plan to test the hypothesis that IL-1 varies on a circadian basis in both human beings and in the laboratory rat. In order to run the human studies a detailed application was prepared and submitted to our Clinical Research Center (CRC) to run these studies. Experiments have been outlined to study circadian variations in body temperature, trace metals, white blood cell concentrations, and IL-1 concentrations in 10 human subjects. We will determine whether the afternoon rise in body temperature in human subjects can be attenuated by administration of aspirin. Each subject will have his/her body temperature recorded on a datalogger (Digistrip II, Kaye Instruments) at 10 minute intervals using thermocouples inserted into the rectum. On day 1 of each experiment, the subject will be given placebos at 4 hour intervals. On day 2 of each experiment, the subject will either be given placebos or aspirin at 4 hour intervals. Blood samples will be taken at 4 hour intervals on both days 1 and 2. The effects of aspirin on body temperature, trace metals, and white cell count will be determined. Plasma concentrations of IL-1 will be determined using the in vitro thymocyte proliferation assay. Prior to testing plasma samples for IL-1 activity, each sample must be gel filtered to remove a large molecular weight inhibitor (Cannon and Dinarello, 1985). Gel columns have been prepared that have been successfully used to separate IL-1 from IL-1 inhibitors and we are now ready to begin these experiments. After consultation with a biostatistician, approval has been granted by our CRC to run these studies.

The animal experiments have involved rats implanted with temperature and activity transmitters. Activity measurement made with the Dataquest System is based upon the principle that as the orientation of the telemetry device (implanted in the rat) changes over the receiver card, the signal strength varies, and hence is picked up as an activity count. We determined the relative hourly activity for each rat by normalizing each hourly activity value to the activity recorded for the hour 11:30 am to 12:30 pm. For example, if the activity value recorded from 2:30 pm to 3:30 pm is 200, and the activity value recorded from 11:30 am to 12:30 pm is 100, then the relative hourly activity from 2:30 to 3:30 pm will be 200/100 or 2.0 (see figure 1).

We have been able to determine the appropriate doses of sodium salicylate that attenuates stress-induced hyperthermia (Singer et al., 1985) and partially attenuates endotoxin-induced fever when these drugs are injected intraperitoneally and intracerebroventricularly. The intraperitoneal administration of an antipyretic dose of sodium salicylate to rats in the late afternoon resulted in significant attenuation of the rise in body temperature normally observed at night; the drug had no effect on activity. These data indicate that the reduction in body temperature cannot be attributed to decreased activity. Administration of this same dose of sodium salicylate to rats during the morning hours had no effect on body temperature, although there were changes in relative activity (see figures 1 and 2). These data will be presented at the FASEB meetings in 1986 (Scales and Kluger, 1986). Experiments are currently in progress to determine whether an intracerebroventricular injection of sodium salicylate and indomethacin (a more potent antipyretic drug) would also block the night-time rise in body temperature. These data suggest to us that the rise in temperature observed at night in rats is a true fever (operationally defined as an elevation in the thermoregulatory set-point), perhaps induced by interleukin-1.

2. Experiments Planned for Year 2:

It is anticipated that the experiments involving circadian variations in body temperature, trace metals, white blood cell counts, and plasma IL-1 concentrations in human subjects will take several months. While these experiments are in progress, we anticipate continuing our animal experimentation. We anticipate completing the studies on the effects of antipyretic drugs injected intraperitoneally and intracerebroventricularly on circadian variation in body temperature. We are also planning to test the hypothesis that the circadian rise in body temperature is a fever by allowing rats to bar press for heat (250 W lamps) in modified Skinner boxes. After rats are trained to press the bar to obtain heat, we will determine whether their preferred environmental temperature (as selected by the rat) increases during the rising phase of their circadian rhythm in body temperature.

Experiments are also planned to determine whether antibodies to IL-1 injected intracerebroventricularly would attenuate the circadian rhythm in body temperature. These studies will be done in collaboration with Dr. John Schmidt of Merck Laboratories (Schmidt et al., 1985). Lastly, we will attempt to determine whether we can detect any circadian changes in plasma and or cerebroventricular concentrations of IL-1. Since many laboratories are working on radioimmunoassays or immunoabsorbant assays for IL-1, presumably more sensitive than the current LAF assay, we may have to wait for the development of these more sensitive assays before a definitive conclusion can be reached regarding circadian variations in IL-1.

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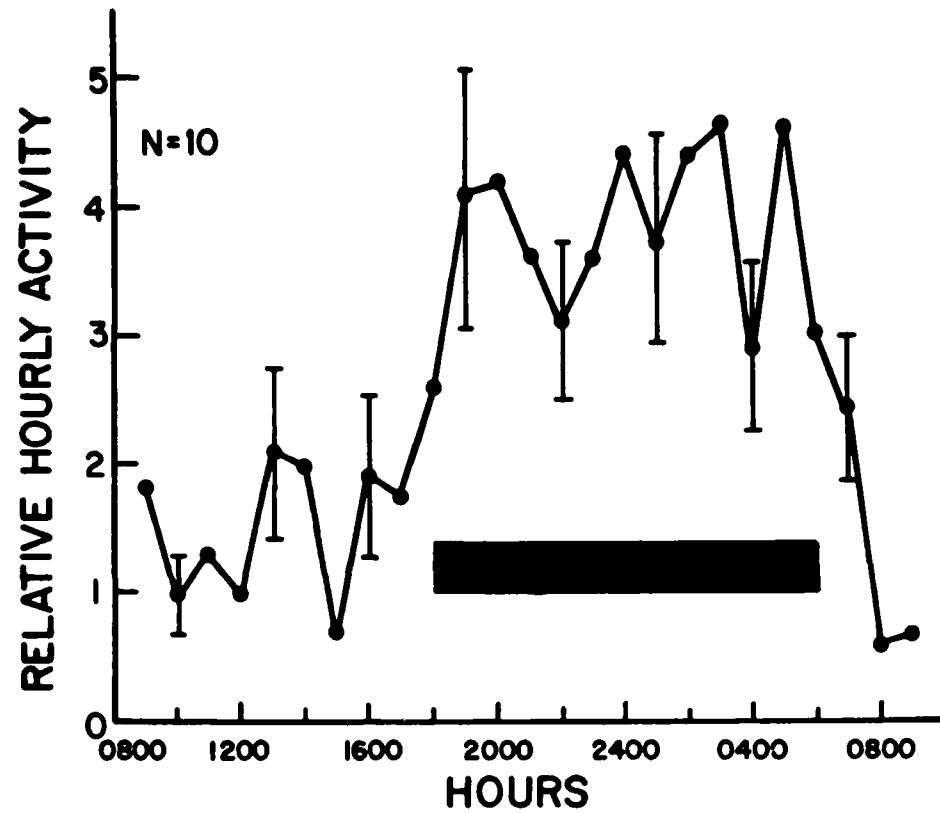


Figure 1. Relative activity of 10 rats. The rats were on a 12:12 light:dark photoperiod; the horizontal bar represents the period of darkness. Each data point represents the mean \pm 1 S.E.

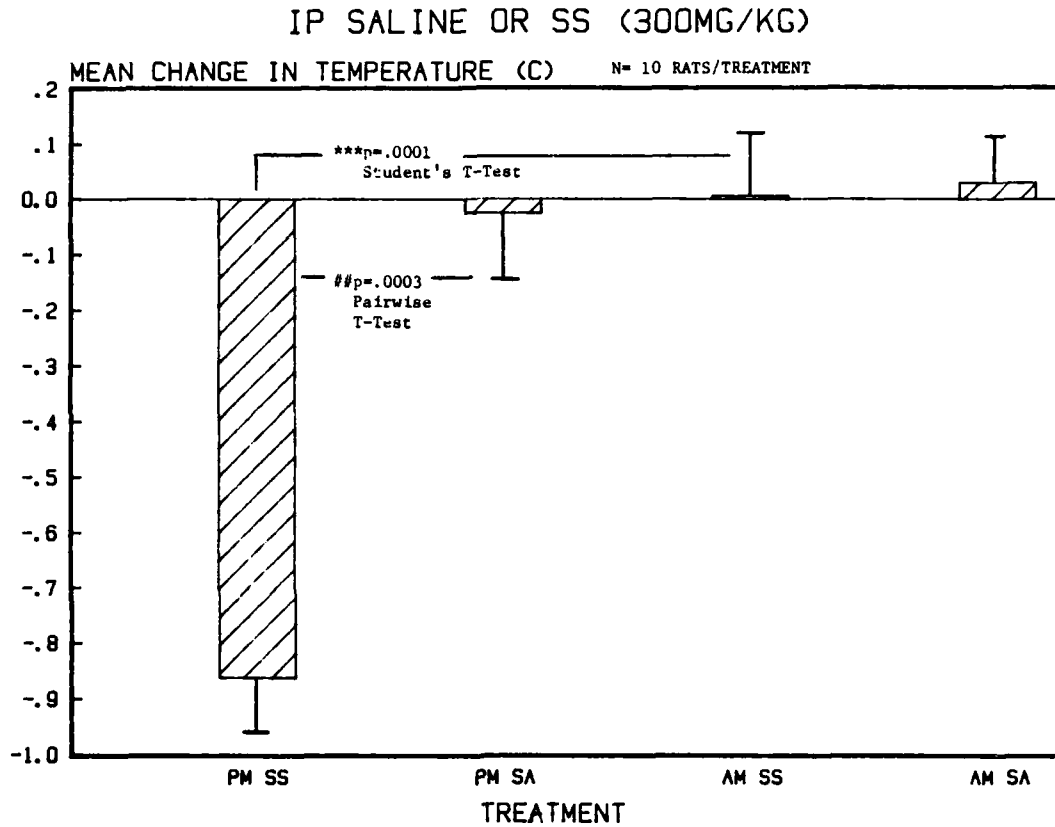


Figure 2. Effect of intraperitoneal injection of 1 ml of 0.9% sodium chloride (SA) or 300 mg/kg sodium salicylate (SS) in 10 rats on the change in body temperature over a 3 hr period following the injection. The injections were given either at 5 PM (during the time of day when body temperature is generally rising) or at 9:30 AM (during the time of day when body temperature is generally low and stable). Comparisons were made between body temperatures on the day of injection and body temperatures on the following day. Only an injection of sodium salicylate in the PM resulted in a significant change in body temperature.

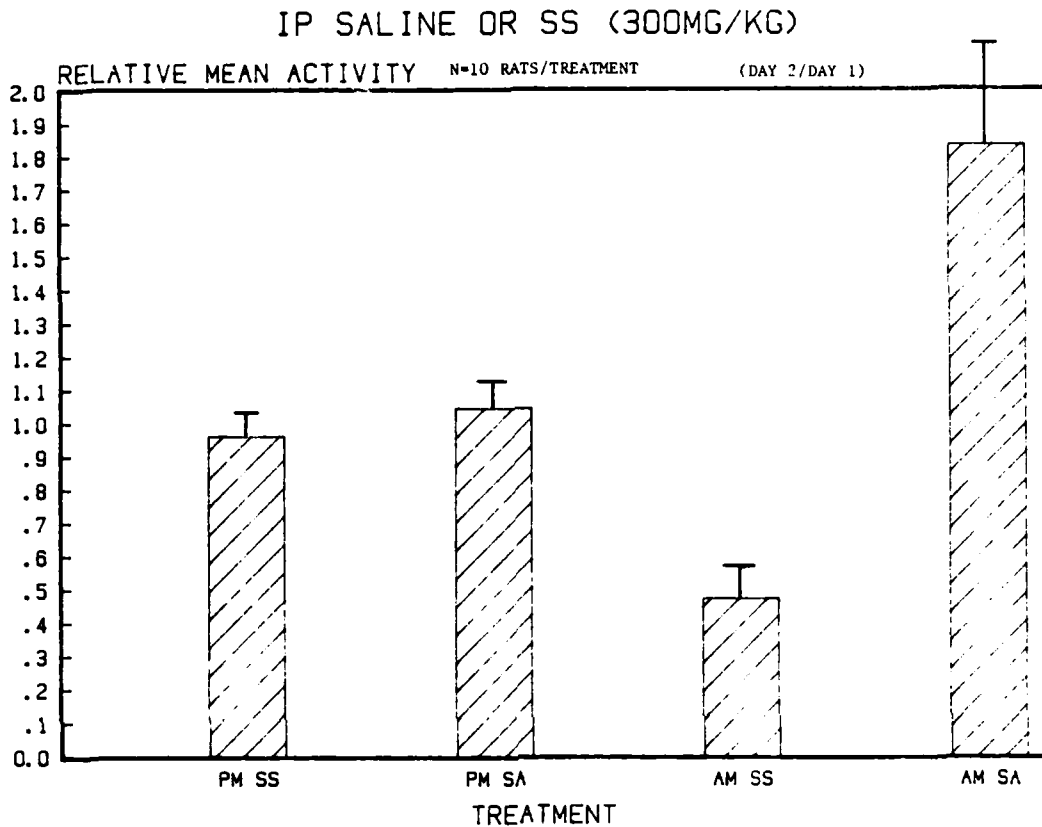


Figure 3. Effect of intraperitoneal injection of 1 ml of 0.9% sodium chloride (SA) or 300 mg/kg sodium salicylate (SS) in 10 rats on relative change in activity. The injections were given either at 5 PM (during the time of day when body temperature is generally rising) or at 9:30 AM (during the time of day when body temperature is generally low and stable). Comparisons were between relative activity on day 1 and the relative activity on day 2 (the day following injection), expressed as a ratio. A value of 1.0 indicates no change in activity; values less than 1.0 indicate greater activity on the day of injection; values greater than 1.0 indicate less activity on the day of injection. These data indicate that although body temperature fell when SS was injected at 5 PM, this was not accompanied by a change in activity. Although an injection of SS at 9:30 AM resulted in an increase in relative activity, this did not result in a change in body temperature. Similarly, although an injection of SA in the AM resulted in a decrease in relative activity, this was not accompanied by any change in body temperature.

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