Alterations in endogenous circadian rhythm of core temperature in senescent Fischer 344 rats

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The data describing the effect of age on the free running, endogenous period of circadian rhythms are also inconsistent. The earliest studies that include aged rats found a significant shortening of free running period of the activity-rest rhythm in the oldest animals compared with "immature" rats (15). Subsequent studies investigating the endogenous period of active wakefulness, core temperature, and drinking behavior in rats support these findings (22, 27). In contrast to the relatively consistent shortening of the circadian period in older rats, the data from mice are ambiguous. The effect of age on circadian period in mice has been reported to shorten (13, 25), remain constant (24), and lengthen (21, 26). Golden hamsters appear to have shorter free running periods of circadian rhythm during aging (20), although in cockroaches, Page and Block (11) report an age-related increase in the period of locomotor activity.

The reasons for differences among reports characterizing age-related alterations in circadian rhythms remain to be elucidated. One possibility for variation in the results may be the use of chronological markers for defining senescence. Median life span has been used extensively as a marker for aging. However, we have shown recently that in male and female Fischer 344 (F344) rats, senescence may be defined by a rapid spontaneous weight loss near the end of life rather than by chronological age (4, 7). Food intake and cold-induced core temperature were not dramatically reduced in older presenescent versus younger (9 mo) rats, but they were in rats that had entered into senescence, defined as the point at which rapid weight loss begins (1, 7). Moreover, the age at which senescence occurred ranged from 24 to 31 mo of age, suggesting strongly that chronological age is not a reliable indicator of this transition into senescence. That circadian rhythm of core temperature (CMT) may decline after transition into senescence rather than as a function of chronological age is consistent with the findings of Li and Satinoff (6). These investigators reported significant variation in the strength and stability of the periodicity of CMT among old female Long-Evans rats ranging in age from 21 to 27 mo. For example, a 25- to 26-mo-old rat had a strong periodicity, whereas a rat of 21 mo did not have a detectable period. When these investigators divided the animals according to the strength of the period, the old "unstable" rats weighed 25 g less than the "good" aged animals.

These studies also suggest that the onset of senescence is determined by the biological, not the precise chronological, age of the animal. The equivocal nature of the data regarding age-related alterations in free...
running circadian rhythms may be due to the use of chronological time, rather than the use of appropriate biological markers as the benchmark for aging. To clarify the relationship among rapid spontaneous weight loss, CRT, and senescence, we reevaluated the effect of aging on endogenous circadian rhythms and the responsiveness of the circadian clock mechanism to light. To this end, we tested two hypotheses: 1) age-related changes in the CRT occur during the same time period as the spontaneous rapid loss in body weight of older rats maintained in constant low red light from 689 days of age until natural death; and 2) the circadian clock, the suprachiasmatic nucleus (SCN) of the hypothalamus, is not functionally sensitive to light input in these senescent animals.

METHODS

Animals and Animal Care

Male F344 rats, aged 4 (122 days; n = 4) and 23 mo (689 days; n = 20), were obtained from the National Institute on Aging colony maintained by Harlan Sprague Dawley Laboratory (Indianapolis, IN). On arrival, animals were housed individually in opaque polycarbonate cages (23.5 x 45.5 x 19 cm) with wood shaving bedding. The rats were maintained at 24–26°C and 50% humidity on a 12:12-h light-dark cycle (lights on at 0600, off at 1800) until the start of the experiment. Rats were provided with NIH-31 laboratory chow (Teklad Research Diets, Indianapolis, IN) and distilled water ad libitum.

Evaluation of Circadian Rhythm of Body Temperature

Five days after arrival, each rat was anesthetized with halothane, and a biotelemetry transmitter (Mini-Mitter, Sunriver, OR) was implanted into the peritoneal cavity via an abdominal incision. After surgery, animals were returned to their home cage and allowed to recover for 3 days. All rats, except one 689-day-old animal, regained at least 95% of the presurgery body weight within this recovery period. The one older rat failing to regain body weight died 10 days after surgery and was eliminated from the data analysis. Four days after surgery, the lighting conditions of the room were changed to constant dim red light (15–20 lx) for measurement of endogenous CRT. Conditions of temperature, humidity, food, and water were the same as described above. The dim red light is perceived as dark by the rat, but allows enough light for workers to perform maintenance duties. Body weights were measured every 3 days, food and water were changed twice weekly, and cage bedding was changed weekly. All work in the animal room was performed at different times each day to minimize the possibility of introducing external time cues and entraining the endogenous CRT. Plots of core temperature of each animal were generated weekly and inspected for possible signs of entrainment and other abnormalities. No entrainment or abnormalities, other than those related to transmitter failure, were noted.

Intraperitoneal temperature was recorded by computer-assisted biotelemetry throughout the experimental period. Every 10 min, temperatures were recorded via computer data acquisition system (Mini-Mitter). For each animal, the system computed the mean and standard deviation of the temperatures collected; temperatures >2 SD from the mean were eliminated, and the mean was recalculated and recorded. The biotelemetry receivers were connected via cable to a computer located outside the room housing the animals.

Intraperitoneal temperature was recorded continuously until the older rats died or until death was imminent (as determined by excessive body weight loss and significant decrease in mean daily intraperitoneal core temperature). Data collection in the younger animals continued until the last older animal died. Average length of life was 629 ± 16 days with a range of 730–943 days. Only rats with complete data sets were used in the analysis. One younger and three older rats were eliminated from the data analysis due to loss of battery power in the Mini-Mitter transmitter during the experiment. One additional older rat was eliminated because it failed to gain weight after surgery. The number of rats used in the final data analysis was 16 old and 3 young.

Data Analysis

The primary objective of the present investigation was to determine if changes in CRT of aging rats occur at approximately the same time as the rapid loss in body weight near the end of life. Because the loss in body weight is independent of the animal’s age, data analysis using chronological age as an independent variable was not possible. That is, the use of a biological marker for age (rapid spontaneous weight loss) precluded analysis of CRT at discrete chronological time points. Thus we divided the CRT data from each older rat into four separate 7-day time intervals: early, middle, presenescence, and senescence. Senescence is defined as the period of rapid spontaneous weight loss. The early period began 2 wk after the start of the experiment; middle represents a period halfway between the start of the experiment and the rat’s natural death; presenescence represents a 7-day period ending 2 days before the start of rapid body weight loss; and the 7-day senescence period began 2 days after the start of rapid body weight loss. Because young rats do not transition into senescence, only three analysis periods were used for them: early, middle and “presenescence” (the term presenescence is used for the young animals only for ease of data presentation; see Table 1). The time periods in the younger rats were matched to the longest-lived rat used in the analysis. The older rats were 319 days of age when they were killed.

The mean core temperature and mean amplitude of the CRT for each rat represent mean daily values averaged over 7 days. Circadian period and strength of the period were determined from a periodogram generated for each 7-day interval. A periodogram is reported as a ratio between the variance across repeated segments of the data and the variance for the entire data set (18). Segments that approximate an inherent periodicity will produce higher variance ratios, seen as peaks in the variance ratio graph.

To illustrate the patterns and relationship among age, body weight, and CRT, we selected three old rats out of a possible sixteen (Figs. 1, 2, and 3). The three old animals were selected to represent different lengths of life and patterns of body weight. Rat A represents a short-lived animal (742 days of age at death), rat B had a life span approximately equal to the group mean (843 days of age at death), and rat C represents a long-lived animal (882 days of age at death).

Responsiveness of the Circadian Pacemaker to Light

The responsiveness of the SCN to light stimulation was examined in six senescent animals. These old animals were killed on the 7th day after showing signs of senescence, as indicated by two consecutive measurements of decreased body mass. Three of the animals were exposed for 60 min to a white fluorescent light source (300 lx) in the first half of their subjective night. The other three animals were similarly killed in the first half of their subjective night without any
white light exposure. After exposure, the rats were anesthetized with Metofane (methoxyflurane), then perfused transcardially with normal saline followed by 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). The brains were removed and placed in fixative at 4°C. Frozen sections of 50 µm through the hypothalamus of all rats were stained immunohistochemically for c-Fos (Onogene Sciences, Cambridge, MA) using the strepavidin-biotin-horseradish peroxidase complex (Abbott, Burlingame, CA) and True Blue (KPL, Gaithersburg, MD) as the chromagen. Sections were mounted on slides, counterstained with neutral red, and secured with a coverslip. The number of immunopositive neurons within the SCN on both sides was counted in each section for every rat.

Statistics

ANOVA was used to determine possible differences among the four analysis periods. When a main effect was found, Fisher’s least-significant difference post hoc test was used to determine differences among the groups. The c-Fos data were compared via a t-test. Differences were considered significant at \( P \leq 0.05 \).

RESULTS

Evaluation of Circadian Rhythm of Body Temperature

Body weights. The age at which body weights of the older rats began to decline rapidly ranged from 714 to 928 days. Although all animals showed spontaneous rapid body weight loss near the end of life, the pattern of body weight loss varied. Fourteen of sixteen older rats used in the analysis had reasonably stable body weights throughout the experimental period, similar to the patterns illustrated in Fig. 1, A and C. A pattern of gradual loss in body weight preceded the rapid spontaneous loss in two of the aged animals, as represented by animal B (Fig. 1B). There was no significant correlation between average body weight before the start of the rapid spontaneous weight loss and the day of the experiment in which rapid weight loss began.

Circadian rhythm of core temperature. Mean core temperature did not differ among the three analysis periods in young rats. Moreover, no significant difference was observed between the young and old rats during the early, middle, and presenescence periods (Table 1). However, mean core temperature of old rats

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<tr>
<td></td>
<td>Early</td>
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<tr>
<td>Young mean core temperature, °C</td>
<td>37.45 ± 0.02</td>
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<tr>
<td>Old mean core temperature, °C</td>
<td>37.35 ± 0.03^a</td>
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<tr>
<td>Young amplitude</td>
<td>0.53 ± 0.02</td>
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<tr>
<td>Old amplitude</td>
<td>0.47 ± 0.01^**</td>
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<tr>
<td>Young period</td>
<td>24.11 ± 0.06</td>
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<tr>
<td>Old period</td>
<td>24.01 ± 0.04</td>
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Values are means ± SE. See METHODS for explanation of analysis period. Term "presenescence" is used for young animals only to maintain consistency in data presentation. Within a row, values sharing or without letter superscripts do not differ significantly. *Value is significantly different from young value within same analysis period (\( P \leq 0.05 \)).

Fig. 1. Body weight (solid lines) and daily mean core temperature (dotted lines) of 3 representative (from a total of 16) male Fischer 344 (F344) rats. Rats were 689 days of age at day 0 of the experiment. A: a short-lived rat (742 days of age at death); B: a rat with length of life approximately equal to the group mean (843 days of age at death); C: a long-lived rat (882 days of age at death). Arrows labeled, early, middle, presenescence (ps), and senescence (s) designate approximate start of that particular 7-day analysis period. A complete explanation of the 4 analysis periods is provided in METHODS.
during the senescence phase was significantly lower than that observed in any of their three nonsenescent time periods. The relationship between body weight and mean core temperature is illustrated in Fig. 1. In general, mean core temperature and amplitude (data not shown) changed significantly 1 day after the onset of senescence. Although there was some variation among the 16 older rats as to the relationship between mean core temperature and body weight, these differences were small.

Two-way ANOVA indicated a significant main effect of age on the amplitude of the CRT (Table 1). That is, CRT amplitude of old rats was significantly less than that of young rats at all time periods. Furthermore, there was a significant decrease in CRT amplitude of the older rats with increasing biological age; the value in the presenescence period was significantly less than that in the early and middle periods, and the value in the senescent period was significantly less than those preceding senescence. Both decreased daily maximum and minimum temperatures (Fig. 2) influenced the significant decline of amplitude in the aging rat at the start of senescence. However, analysis of rate of decline indicated that the maximum core temperature declined significantly faster than the minimum daily core temperature.

All older rats exhibited strong CRT before senescence (Fig. 3), but after the onset of senescence, CRT varied considerably. The rapid change in CRT between presenescence and senescence is further illustrated in the periodogram plots for these animals (Fig. 4). Although the period did not differ significantly between presenescence and senescence (Table 1), the strength of the period, as determined by periodogram analysis, was altered significantly in the senescent versus presenescence animals.

**Light Sensitivity of the SCN**

Examination of light-induced c-fos expression in the SCN of senescent animals demonstrated a clear difference between the light-exposed and non-light-exposed animals. Photomicrographs (Fig. 5, A and B) of the SCN from one rat in each group illustrate the lack of effect of senescence on c-Fos production after a light pulse. After the onset of senescence, the light-exposed rat (Fig. 5B) exhibited a greater number of c-Fos-reactive neurons within the SCN than did the non-light-exposed rat (Fig. 5A). The summary response of all six animals (Fig. 5C) illustrates the significant difference
(P < 0.001) in the mean number of c-Fos-reactive SCN neurons between non-light-exposed rats (26 ± 4) and the rats exposed to light (4,126 ± 336).

DISCUSSION

Our previous investigations demonstrated that dramatic age-related alterations in cold-induced thermoregulation, food intake, and adrenergic responses of isolated mature brown adipocytes are associated with the transition to a physiological state that can be identified by the animal’s spontaneous rapid weight loss (1, 4, 7). These events occur near the end of life rather than at a specific chronological age. We now extend these observations to include endogenous circadian rhythm of core temperature. Characteristics of the endogenous circadian pattern, including daily mean core temperature, amplitude, and strength of the period in senescent rats maintained in dim red light were significantly different from values obtained during periods of stable body weight in the same rats. Only the amplitude of the CRT differed significantly between weight-stable aged and younger rats. These data are consistent with our previous observations on the timing of age-related functional loss and suggest strongly that the use of chronological time as a marker for senescence is not tenable.

The present data confirm previous findings that age-related alterations in several physiological systems occur at about the same time point in the life span of the rat. Other investigations report that rhythmicity of various functions in humans and laboratory animals (i.e., sleep-wake cycle, core temperature, locomotor activity, and drinking behavior) tend to decline with age at similar times and rates (6, 9, 17). The observation that circadian rhythms show comparable patterns of age-related loss suggest altered hypothalamic regulation of these functional declines (10). Our present and previous data indicate that age-associated hypothalamic dysfunction includes noncircadian as well as circadian variables. For example, age-related attenuation in cold-induced thermoregulation and aspects of energy balance (food intake + body weight), two systems that involve hypothalamic regulation, occur at times similar to that seen in changes of the circadian rhythm of core temperature (7). Functional loss proceeds rapidly, in some cases within 12 h of the last
“normal” recorded value. Furthermore, recent observations suggest that altered metabolism also occurs in isolated cells from senescent rats (4). Maximal norepinephrine-stimulated oxygen uptake of mature brown adipocytes isolated from male and female rats experiencing spontaneous rapid weight loss was significantly less than that observed in cells isolated from age-matched, weight-stable rats, including those induced to lose weight via food restriction (4). These data indicate that the alterations occurring during senescence may include physiological systems that are distal to, but influenced by, the hypothalamus.

Age-related disruption in the circadian pattern can result from alterations in the mediators that regulate the overt rhythm, from changes occurring directly within the pacemaker mechanism, or from a combination of the two. Evidence for each of these possibilities exists, but definitive conclusions have not been drawn. Decreases in daily mean amplitude of core temperature observed here during the rapid weight loss phase and reported extensively in the literature (see review Ref. 14) suggest an age-related alteration in the interaction between the pacemaker mechanism and the overt rhythm. Conversely, loss in the strength of the endogenous period (see Fig. 4) implies disruption to the pacemaker itself. Supporting evidence for the latter includes age-related lesions to the SCN (5) and restoration of corticotropin-releasing hormone rhythm of aging rats through implantation of fetal grafts containing the SCN (3). Moreover, Satinoff and colleagues (17) found that neural firing rates of brain slices containing the SCN were correlated to the pattern of CRT in young, but not old, rats. These investigators concluded that aging most likely affects the endogenous rhythmicity via the SCN itself. However, the ability of light to elicit c-fos expression in the SCN of senescent rats (Fig. 5) clearly indicates that this region remains capable of responding to environmental input. This observation is consistent with the results of Sutin et al. (19), who report that c-fos mRNA levels increase significantly after a light pulse in 22-mo-old male F344 rats adapted to constant dark. Together, these data suggest that if there is an age- or senescence-related deterioration of the circadian pacemaker mechanism it most likely occurs downstream to this level of processing. However, additional studies are warranted to more precisely define the location of the possible alteration.

The lack of congruency among investigations with respect to the location of age-related alterations to circadian pattern may reflect, in part, the physiological state of the animals at the time the measurements were made. As seen in the present investigation, amplitude of the CRT in weight-stable older rats is significantly less than that observed in the younger animals. This implies alterations in a component(s) that contributes to the overt rhythm. However, the strength of the period, one measure of pacemaker functionality, is not altered in the older rat until rapid spontaneous weight loss occurs. This finding is consistent with the data of Li and Satinoff (6), who report that a diminution of the CRT period occurs in older rats that have lost body weight. Their data (6) and those presented here are compelling evidence supporting the suggestion that biological markers, rather than chronological time, must be used to investigate the effect of aging on circadian function.

The finding that endogenous amplitude and period become altered at different times and decline at different rates in the life span of the rat is consistent with our previous data describing at least two rates of body weight loss during aging (4, 7). The decline in amplitude appears to occur slowly and without a dramatic decrease in physiological function, a finding similar to that observed during the weight-stable period where, in fact, a small decrease in body weight may occur. However, with the sharp decline in body weight during senescence, there occurs a rapid disruption in the endogenous period concomitant with greatly attenuated physiological functioning. Although the mechanisms that account for this age-related variance in the timing of circadian patterns have yet to be elucidated, we speculate that they represent measures of changes to integrated systems as opposed to a direct measure of the controlling mechanism. The measures of amplitude and mean core temperature reflect more closely the function of an integrated system that includes controls for blood flow, vasodilation, and metabolic rate. It is possible that diminution in one of these systems may be masked by compensatory improvements of another, resulting in only gradual or no significant change in the maintenance of homeothermy. Indeed, previous research in humans and laboratory rodents has shown that small and gradual age-related losses in thermoregulation may involve such compensation (8, 23). Because there are no “backup” systems that can compensate for alterations in the clock mechanism, any age-related change to the clock will result in rapid and immediate alterations in the circadian pattern, as seen in the periodogram analysis (Fig. 4). Together, these data suggest that age-related alterations of circadian pattern are related directly to the control mechanism, i.e., the clock and its efferent pathways, rather than components of the overt rhythm.

In summary, our previous investigations have shown that that aging F344 rats undergo a transition from aging to senescence that is associated with a rapid decline in food intake and body weight, development of severe, cold-induced hypothermia, and alteration in peripheral cellular responses. This study demonstrates disruption in the endogenous circadian rhythm of temperature. The rapid disruption in the endogenous circadian period of the senescent animals suggests strongly that alterations occur predominantly in the clock mechanism rather than in components of the overt rhythm. However, the fact that c-Fos activation still occurs in response to environmental input (i.e., light) indicates that the decrement in rhythms may be in the efferent portion of the circadian pacemaker. The functional declines in the circadian system were not associated with chronological age, as the transition into senescence occurred in animals ranging in age from 714 to 928 days. Our data and those of Li and Satinoff (6)
support the contention that biological markers must be used to determine precisely the effects of aging on circadian timing and possibly other systems as well.

Perspectives

The data presented here are consistent with our previous investigations suggesting an altered physiological state that is characterized by diminished physiological function near the end of life and that is not associated with a common pathology (1). The rapid decline in physiological function observed in the senescence F344 rats, i.e., diminished food intake, cold-induced thermoregulation, and circadian rhythm, concurrent with rapid loss in body weight (Ref. 7; Table 1) is similar to the functional decline observed in humans diagnosed with geriatric failure to thrive (16). Geriatric failure to thrive is a syndrome characterized by unexplained attenuated appetite, rapid weight loss, impaired immune function, and disruption in several other physiological systems (12). Although the etiology of this syndrome is unknown, it has been proposed that the symptoms of failure to thrive are simply manifestations of a prelude to “natural” death. Failure to thrive presupposes that the origins of this syndrome are predominantly biological rather than pathological. Our previous and present data suggest that the origin of this biological change involves the hypothalamus and/or effector pathways associated with this brain region. Thus we believe that this model of rapid senescence can provide valuable insight into possible hypothalamic involvement in the regulation of aging as well as determine mechanisms that may reflect geriatric failure to thrive.

The authors thank Carol Murtagh-Mark, Craig Boon, and Yan Wang for technical assistance. The authors thank Jack Hamilton and Dr. I.-Hsiung Tang for helpful comments.

This study was supported in part by National Institutes of Health Grants AG-06665, DK-35747, and HL-53205; National Aeronautics and Space Administration Grant NAG2-944; and a gift from the California Age Research Institute.

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Received 3 August 1998; accepted in final form 3 December 1998.

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