Ingestive behavior and body temperature during the ovarian cycle in normotensive and hypertensive rats

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Received 18 October 2000; accepted in final form 17 September 2001

Rashotte, Michael E., Allison M. Ackert, and J. Michael Overton. Ingestive behavior and body temperature during the ovarian cycle in normotensive and hypertensive rats. Am J Physiol Regulatory Integrative Comp Physiol 282: R216–R225, 2002; 10.1152/ajpregu.00676.2000.—The relationship between ingestive behavior (eating + drinking) and core body temperature (Tb) in naturally cycling female rats was compared in a normotensive strain (Sprague-Dawley; SD) and a hypertensive strain reputed to have chronically elevated Tb (spontaneously hypertensive rats; SHR). Tb (by telemetry) and ingestive behavior (automated recording) were quantified every 30 s. Ingestive behavior and Tb were related on all days of the ovarian cycle in both strains, but the strength of that relationship was reduced on the day of estrus (E) compared with nonestrous days. Several strain differences in Tb were found as well. In SHR, dark-phase Tb was elevated on E, whereas SD remained at the lower nonestrous values. Fluctuations in dark-phase Tb were correlated with ingestive behavior in both strains but had greater amplitude in SHR except on E. Short-term fasting or sucrose availability did not eliminate elevated dark-phase Tb on E in SHR. We propose that estrus-related changes unique to SHR may indicate heightened thermal reactivity to hormonal changes, ingestive behavior, and general locomotor activity.

spontaneously hypertensive rat; Sprague-Dawley rat; estrus; telemetry; feeding; drinking; fasting; sucrose

THE RELATIONSHIP between ingestive behavior (eating and drinking) and body temperature (Tb), of interest for decades (1, 3, 4, 15), has been studied in several species. For example, brain temperature in rats and cats begins to increase a few minutes after eating or drinking is initiated (16), skin and liver temperature become elevated during eating in rats (7), and skin temperature in the vicinity of the liver increases in normal-weight humans during consumption of a meal (32). Ingestive behavior and Tb have separately been shown to vary across days of the ovarian cycle, but there is no information about the relationship between the two measures during the cycle. The present study, which characterizes the relationship between ingestive behavior and Tb in naturally cycling female rats from two strains, provides the first data on whether the relationship is constant during the cycle and whether it is similar in different strains.

It is well known that the ovarian cycle influences ingestive behavior in rats of several strains. Daily intake of food and water is relatively invariant on the preestrous days of a typical 4-day cycle (diestrus 1 (D1), diestrus 2 (D2), and proestrus (P)), but on the day of estrus (E) intake of both food and water, it decreases (5, 9, 11, 29, 31). The decrease in food consumption on E is always associated with a decrease in the size of individual feeding bouts, but the frequency of bouts has been reported to increase on E (2, 9, 19, 30) or to remain the same as on other days in the cycle (8, 10). Changes in drinking-bout parameters associated with decreased water intake on E have not been reported.

Ovarian-cycle influences on the daily Tb rhythm in rats are less well characterized, primarily because few studies have used telemetric methods that permit Tb to be recorded frequently during the day without disturbance to the rat. Such methods have been used to obtain data on abdominal Tb in two rat strains. In one case, Holtzman rats housed in standard laboratory cages showed higher Tb in the dark phase of P when Tb was recorded six to nine times per day (28, 34). More detail of the daily Tb rhythm during the ovarian cycle was provided in a study with female Sprague-Dawley (SD) rats of the Charles River strain CD, whose Tb was recorded every 10 min (18). In this case, the rhythm was shown to depend on housing conditions: rats with access to a running wheel showed elevated Tb in the dark phase of P and in the light phase of E; in rats that could not run, however, Tb was elevated in the dark and light phases of E, and there was a sawtooth pattern in dark-phase Tb in some rats, characterized by large-amplitude fluctuations of ~1°C, particularly on nonestrous days of the cycle (18). Because rats are more active and eat and drink mostly in the dark phase (27), it is possible that this sawtooth pattern in Tb is closely related to episodes of ingestive behavior.

In the present study, we first characterize the relationship between ingestive behavior and Tb during the natural ovarian cycle in SD rats and in spontaneously hypertensive rats (SHR). SHR have the interesting...
feature that their phenotype may include chronically elevated Tb compared with the normotensive strains such as SD (see Refs. 6 and 22). To achieve high detail in our measurements, ingestive behavior and Tb (abdominal) were quantified in 30-s bins. Because both strains were housed in standard laboratory cages, we expected a sawtooth pattern in Tb on nonestrous days, as previously reported for SD rats in similar housing conditions (18), and we predicted that peaks in the sawtooth pattern would be closely related to ingestive activity as suggested by studies showing elevated Tb during eating. Because hormonal changes surrounding E are thermogenic (13, 20, 23), we also predicted that the relationship between ingestive behavior and Tb may be weakened on E.

In a second part of the experiment, we investigated the relationship between ingestive behavior and Tb during the ovarian cycle in SHR by manipulating ingestive behavior in different parts of the cycle. In one manipulation, we prevented eating by removing food; in another, we enhanced drinking by making a palatable sucrose solution available. Each manipulation was imposed for 2-day periods, separately in the early and the late parts of the ovarian cycle. Because the regularity of the ovarian cycle is known to be differentially affected by alterations in dietary fuel availability early or late in the cycle, at least in hamsters (21, 25), we also assessed how cycle regularity was affected in SHR after the fasting and high-energy sucrose treatments were given.

**METHODS**

**Animals**

Female rats from the SHR (n = 24) and SD (n = 7) strains were purchased from Harlan Sprague Dawley (Indianapolis, IN). The rats were ~3 mo old at the start of data collection when average (±SE) body weight was 204.3 ± 0.8 g for the SHR and 261.3 ± 2.0 g for the SD strain. A larger number of SHR were used to stock subgroups that received counterbalanced treatments when fasting, and high-calorie exposures were imposed in a second part of the experiment. The ovarian cycles of each rat were characterized with the vaginal-smear method described below. Rats were selected for data collection that had at least two repeating cycles in succession: 19 SHR had repeating 4-day ovarian cycles (D1, D2, P, E); 6 SD rats had repeating 5-day cycles in which there was an added day of diestrus (D1, D2, D3, P, E). The procedures were approved by the Animal Care and Use Committee at Florida State University.

**Apparatus**

Rats were housed individually in hanging cages (floor surface: 20.3 × 24.8 cm; height 17.8 cm) in a room where ambient temperature was 23 ± 1°C and a 12:12-h light-dark cycle was in effect (lights on at 0700). Purina 5001 powdered chow (13.8 kJ/g) and deionized water were available ad libitum from a spill-resistant feeder and a water bottle located on opposite walls of the cage. A daily maintenance procedure occurred ~2 h after lights on, during which the rats were removed from the cages and weighed, vaginal smears were taken, and food and water were weighed and replenished. Food spillage associated with each cage was easily identified, measured, and taken into account when daily food intake was calculated. The feeder and bottle were instrumented and connected to a computer to measure the time each rat spent eating (1-s resolution) and licks on the water bottle tube in successive 6-s bins throughout the day (see Ref. 27 for details). By means of a calculation that assumed licks occurred at six per second (17), the original drinking measure (licks/6 s) was converted to time spent drinking (1-s resolution) to match the units of measurement for eating. In the second part of the experiment, a bottle containing sucrose solution was added on some days. That bottle was not instrumented to record licking behavior. The roof of each cage contained an antenna to detect pulses per second from the abdominal Tb transmitter. Feeding, drinking, and Tb data were measured continuously and saved for offline analysis.

**Ovarian Cycles**

Ovarian status was monitored daily by examining vaginal smears under a low-power microscope. Smears were obtained by inserting a drop of water from a pipette into the vagina and removing a small sample of cells from the vaginal walls. Standard criteria were used to characterize the days of the cycle (12). Smears were made during the daily maintenance period 2 h after lights on; the ovarian phase identified by each smear was assigned to the preceding 24-h period. As a result, E included the luteinizing hormone surge, ovulation, and the entire nocturnal period during which female rats are sexually receptive (12).

**Tb**

The selected rats had a temperature-sensitive radio transmitter (Barrows, Magalia, CA; model T; 37°C, ~550 Hz) implanted in the abdomen, with no special provision for fixing its location, while anesthetized with a mixture of ketamine (73 mg/kg) and xylazine (8.8 mg/kg). The time constant of the transmitters was 1.4 min.

**Experimental Protocols**

*Between-strain comparison of Tb rhythms and ingestive behavior.* After implantation of the Tb transmitter, SHR (n = 19) and SD rats (n = 6) were returned to the test cages and allowed to recover for at least 8 days, or until two normal ovarian cycles were completed in succession. At that time, Tb and feeding behavior were measured for three additional consecutive ovarian cycles, which provided the data for analysis to determine strain effects on the relationship between ingestive behavior and Tb across the ovarian cycle.

*Within-strain (SHR) manipulation of ingestive behavior.* Immediately after the between-strain protocol was completed, SHR (n = 19) continued testing to evaluate how average dark-phase Tb in this strain is affected by experimental manipulation of ingestive behavior and energy availability on the first 2 days or last 2 days of the ovarian cycle. All interventions began 2 h before lights off, when the appropriate change in food or fluid availability was made in the cages, and ended 48 h later. The first manipulation, fasting, eliminated feeding activity. The rats were randomly assigned to one of two subgroups that differed in the order two 48-h fasts were administered. Rats in one subgroup (n = 10) were initially fasted on D1 and D2 of their cycles, and after a recovery period of at least 8 days in which food and water were available ad libitum and all measures returned to baseline levels, they were fasted on P and E followed by an 8-day recovery period. The other subgroup (n = 9) received the fasts in the opposite order.
After the second recovery period, the rats were further tested to evaluate how dark-phase Tb is affected by a 48-h period of enhanced drinking activity resulting from the availability of a bottle containing sucrose solution (320-g reagent-grade sucrose added to each liter of deionized water; 26.5% sucrose by weight) along with regular food and water. The same subgroups received 2-day periods of sucrose availability on D1-D2 and on P-E in a counterbalanced order separated by 8-day recovery periods, as was done in fasting. Intake of sucrose solution was measured every 24 h by weighing the bottle. Energy consumed from the solution (4.04 kJ/g) and food (13.8 kJ/g) was calculated each day.

Response Measures

Tb. The Tb transmitter output (counts per second) was averaged into 30-s bins and converted to degrees Centigrade values (0.001°C resolution) for use in several analyses. A mean value of Tb for the dark and light phases was also calculated for each day of the cycle. In this calculation, 1) phase means were computed for each rat on each day of the three ovarian cycles when measurements were made, 2) for each rat, single means for the light and dark phases on each day of the cycle were computed by averaging the appropriate three means, and 3) group averages were calculated using individual rat data from 2. Dark-phase means were based on 12 h of data (1,440 30-s bins per rat per phase); light-phase means were based on the last 6 h of the phase (720 bins) to avoid the influence of handling and the vaginal-smear procedure at daily maintenance.

Food and water intake. Food and water intake were measured daily, and average values for each day of the ovarian cycle were calculated by combining daily data from individual animals in the manner described above for average Tb.

Ingestive behavior in dark phases. The separate times spent eating and drinking per 6-h bin were converted to 30-s bins and summed to provide the total ingestive time per bin (maximum = 30 s). The larger bin size for ingestive behavior matched the 30-s bin size for Tb and facilitated several statistical comparisons between ingestive behavior and Tb. Ingestive behavior was also subjected to a bout analysis. On each day, bouts of ingestive behavior were identified in the dark phase by the following criteria: 1) the minimal requirement was at least 3 s of ingestive activity in a bin, followed by some ingestive activity in at least 1 of the next 10 bins; and 2) bouts ended in the bin that was followed by 10 consecutive bins without ingestive activity. These criteria excluded from bouts the rare occurrences of ingestive activity in an isolated single bin. The descriptive statistics for each bout were (30-s resolution) 1) the times in the dark phase at which the bout began and ended, 2) bout duration (ending bin time minus starting bin time), 3) percentage of the bout occupied by ingestive activity (cumulative time spent eating or drinking per bout/total bout duration × 100), and 4) interbout interval (bout n + 1 starting bin time minus bout n ending bin time).

Statistical Analysis

Between-strain comparisons were made with a commercial statistical package using two-way repeated-measures ANOVA and Tukey post hoc pairwise comparisons (Sigma Stat 2.0, SPSS, Chicago, IL). Statistical significance of P < 0.05 was accepted. Pearson correlations and cross-correlations were calculated with a statistical add-in for Microsoft Excel (WinSTAT, R. Fitch Software, http://www.winstat.com). To allow between-strain comparisons of days in the ovarian cycle, the 5-day cycles in SD rats were converted to 4-day cycles by dropping the extra diestrous day (D3) that occurred in SD rats. Tb and ingestive behavior measures on D3 were similar to those obtained on D2 in SD rats.

The effects of fasting and of exposure to sucrose solution were assessed by comparing mean dark-phase Tb for each subgroup on days when diet was manipulated against comparable control days for those subgroups in ovarian cycles when food and water were available ad libitum. Because the order in which each manipulation was imposed in different parts of the ovarian cycle had no statistically significant effect, data from the counterbalanced subgroups were combined for the analysis. Statistical comparisons were made by repeated-measures ANOVA and post hoc pairwise comparisons (Tukey). Repeatability of the ovarian cycle after each manipulation was assessed by χ² tests of the percentage of animals whose ovarian smears did not deviate from the regular 4-day pattern (D1, D2, P, E) during the 8-day recovery period that followed the cycle in which fasting or exposure to sucrose solution occurred.

RESULTS

An example of eating, drinking, and Tb data during the ovarian cycle recorded from an individual rat of each strain is shown in Fig. 1A (SHR-5) and Fig. 1B (SD-2). Several features of these data highlight points of special interest in the analyses of grouped data that follow: 1) on D1, D2, and P, there is a sawtooth pattern in dark-phase Tb in both rats, and greater amplitude in the pattern is indicated in SHR-5; 2) high peaks in Tb are generally associated with episodes of ingestive behavior in both rats; 3) the amplitude of the sawtooth pattern decreases on E in SHR-5, primarily because low values in the pattern were moderated; and 4) ingestive behavior becomes more continuous on E in both rats.

Tb During the Ovarian Cycle

Averaged data for each strain (Fig. 2A) indicate that across the ovarian cycle, dark-phase Tb was relatively stable except in SHR for which Tb increased on E (by 0.31°C, relative to D1). ANOVA yielded only a significant change in dark-phase Tb across days (F = 10.81, df = 3,69; P < 0.001) and a strain × day interaction (F = 18.81, df = 3,69; P < 0.001). Post hoc comparisons indicated that 1) the strains differed only on E (P < 0.001), 2) SHR Tb was significantly higher on E than on any other day of the cycle (P < 0.001), which did not differ among themselves, and 3) SD Tb was not significantly different across days of the cycle. Analysis of light-phase Tb indicated significant change across days of the cycle (F = 11.32, df = 3,69; P < 0.001) and a strain × day interaction (F = 3.63, df = 3,69; P = 0.02). Pairwise comparisons showed that, in each strain, light-phase Tb on P was significantly lower than on some other days in the same cycle: P was lower than D2 in SHR (P < 0.01) and P was lower than all other days in SD rats (P < 0.03). In SHR, light-phase Tb was also lower on E than on D1 and D2 (P ≤ 0.006). The significant strain × day interaction likely reflects the difference at P where the largest mean difference between strains occurred (SD rats were 0.16°C below SHR). In the pairwise comparisons, P = 0.09 for this difference.
We quantified and analyzed the sawtooth pattern in Tb that was evident in the individual animal data of Fig. 1. A standard deviation for Tb values in the 30-s bins of each dark phase of the ovarian cycle was calculated for each rat, and the average standard deviations for the two strains are shown in Fig. 2B. ANOVA of these data yielded a strain difference (F = 18.57, df = 1,23; P < 0.001), a day effect (F = 12.85, df = 3,68; P < 0.001), and a strain × day interaction (F = 4.02, df = 3,68; P = 0.01). Post hoc tests indicated that the standard deviation was significantly higher for SHR than for SD on D1, D2, and P (P ≤ 0.003) but that no difference attributable to strain occurred on E. Further post hoc tests indicated that, for SHR, the standard deviation of Tb was significantly lower on E than on all other days (P < 0.001) and was significantly lower on P than on D1 and D2 (P < 0.05); for SD rats, there was no significant difference in standard deviation of Tb among days. These results support the impression given by the individual animal data in Fig. 1 that the sawtooth pattern in Tb has greater amplitude in SHR than in SD rats on D1, D2, and P and also that, in SHR, the oscillations in Tb become dampened on E.

Changes in the amplitude of the sawtooth pattern in Tb across the ovarian cycle were assessed by calculating the highest and lowest values of Tb in each dark phase. For each rat, thirty 30-s bins in each dark phase with the highest Tb values were identified, and a mean high-Tb was computed; the same procedure was used to identify the lowest Tb values in the phase and to calculate the mean low Tb. The resulting data were averaged for each strain and plotted in Fig. 2C.

Fig. 1. Illustrative relationships between ingestive behavior and core body temperature (Tb) on each day of the ovarian cycle for individual spontaneously hypertensive rat (SHR-5; A) and Sprague-Dawley rat (SD-2; B). Data are plotted in 30-s bins; dark phases are indicated by closed bars at top of each panel. Plots of eating or drinking show the number of seconds in each 30-s bin that a photobeam at the feeder or water bottle was interrupted; each response is plotted from a common zero x-axis to facilitate presentation of the results. No axis is shown for the eating and drinking plots, but the maximum (30 s) activity time in each bin is reached in some of the records. The period when Tb data were interrupted while the rats were removed from their cage for daily maintenance (around the 2nd h of the light phase) is evident in some records. Tb and ingestive activity were often elevated for a short period after maintenance. Cycle days: D1 and D2, diestrus 1 and 2; P, proestrus; E, estrus.
ANOVA of the high-Tb values yielded a significant effect of strain (F = 6.23, df = 1,23; P < 0.05) and day (F = 2.87, df = 3,68; P < 0.05), indicating that SHR had significantly higher high-Tb values on all days of the cycle, and post hoc tests indicated that values on P were significantly lower than on D1 (P < 0.05). The analysis of the low-Tb values yielded a day effect (F = 11.05, df = 3,68; P < 0.001) and a strain × day interaction (F = 6.84, df = 3,68; P < 0.001). Post hoc tests indicated that in the early part of the ovarian cycle, SHR had lower low-Tb values than SD rats (P < 0.05 on D1) but that on later days the values for SHR increased such that low Tb was significantly higher on P than on D2 (P < 0.05) and on E than all other days (P < 0.001). The average low Tb on E was 0.52°C higher than on D1 in SHR. In contrast, post hoc tests for SD rats indicated no significant change in low Tb across days of the ovarian cycle. The results of this analysis support the impression given by the individual rat data in Fig. 1 that, in SHR, the low values of dark-phase Tb are higher on E than on other days of the cycle.

Food and Water Intake During the Ovarian Cycle

Significant changes in food and water consumption occurred across days of the ovarian cycle, with reduced consumption on E being the consistent result in both strains (Fig. 3A). ANOVA of daily food intake yielded only an effect of days (F = 21.74, df = 3,69; P < 0.001), and pairwise comparisons indicated that less food was eaten on E (mean 14.3 g; P < 0.001) than on the other days, which did not differ among themselves (mean 17.5 g). The analysis of daily water intake yielded a significant difference between strains (F = 22.50, df = 1,23; P < 0.001), and pairwise comparisons indicated that SD rats drank more water than SHR did on each
day of the cycle ($P \leq 0.004$). On D1, for example, SD rats consumed 10.7 g more water than SHR; on E, the difference was 6.1 g. There was also a significant change in water intake across days of the cycle ($F = 23.98, \text{df} = 3.69, P < 0.001$) and a strain $\times$ day interaction ($F = 3.47, \text{df} = 3.69, P = 0.02$). Pairwise comparisons indicated that each strain drank significantly less water on E than on the other days of the cycle ($P \leq 0.03$) and also that SD rats drank less water on P than on D1 ($P = 0.04$).

**Ingestive Behavior in the Dark Phases of the Ovarian Cycle**

Because most ingestive behavior occurred in the dark phase (Fig. 1), we characterized the features of ingestive behavior in that phase by analyzing the behavior into bouts using the criteria described earlier. Figure 3B shows that there were $\sim 15$ bouts of ingestive behavior per day on D1, D2, and P but $\sim 23$ bouts per day on E. ANOVA of these bouts per day data yielded only a significant effect of day ($F = 49.28, \text{df} = 3.67, P < 0.001$), which post hoc tests indicated was attributable to the large increase on E ($P < 0.001$). Figure 3C shows that ingestive bouts averaged $\sim 11$ min in duration on D1, D2, and P and that they became much shorter on E (6.5 min).

ANOVA of the bout duration data yielded a strain difference ($F = 6.44, \text{df} = 1.23, P < 0.05$) that reflected somewhat longer bouts in SHR when data from all days are collapsed. There was also a day effect ($F = 21.67, \text{df} = 3.67, P < 0.001$) but no strain $\times$ day interaction. Post hoc tests indicated that bout duration on E was significantly shorter than on all other days of the cycle ($P < 0.001$). The interbout interval (Fig. 3D) averaged $\sim 40$ min on D1, D2, and P but decreased to 25.6 min on E. ANOVA yielded only a significant effect of day ($F = 25.92, \text{df} = 3.67, P < 0.001$); post hoc tests indicated that interbout intervals on E were lower than on all other days in the cycle ($P < 0.001$) and also that intervals on P were shorter than on D2 ($P < 0.05$). In data not shown here, we found that, on average, each strain spent $\sim 40\%$ of the total bout time engaged in ingestive behavior on each day of the ovarian cycle. ANOVA of this measure yielded no significant effect for strain or day.

**Relationship Between Ingestive Behavior and $T_b$ in the Dark Phases of the Ovarian Cycle**

The relationship between ingestive activity and $T_b$ in the dark phases of the ovarian cycle was initially analyzed by calculating a Pearson correlation coefficient for each rat on each day of the cycle. In this calculation, time spent in ingestive behavior in each 30-s bin of the dark phase was correlated with the value of $T_b$ in that bin. Computer-file characteristics resulted in there being 1,424 pairs of values in SHR correlations and 1,404 pairs in SD correlations. The resulting correlation values were then averaged, plotted for each strain (Fig. 4A), and subjected to ANOVA. There was only a significant effect of day in the analysis of these correlation coefficients, and post hoc tests indicated that the correlation between ingestive behavior and $T_b$ was lower on E than on other days of the cycle ($P < 0.001$), which did not differ among themselves. A subsequent analysis indicated that the correlations were calculated on pairs of values for which the percentage of pairs that included ingestive behavior (i.e., values $>0$ in the ingestive-behavior member of the pair) was higher in SHR and, in that same strain, varied across days of the cycle: the mean percentages, collapsed across D1, D2, and P, were 20.19% for SHR and 13.16% for SD rats; on E, the values were 16.81% for SHR and 12.76% for SD rats. ANOVA and post hoc tests indicated that SHR had higher percentages than SD rats on every day of the cycle and that SHR alone had reduced percentages on E ($F_{\text{strain}} = 37.27, \text{df} = 1.23, P < 0.001; F_{\text{day}} = 3.83, \text{df} = 3.69, P = 0.01; F_{\text{strain} \times \text{day}} = 3.40, \text{df} = 3.69, P = 0.02$). Taken
together, these findings indicate that the percentage of bins with above-zero values for ingestive behavior is unrelated to the reduced correlation found between ingestive behavior and T_b on E.

The correlation data in Fig. 4A were obtained when values of ingestive activity and T_b from the same time bin were paired (zero-lag correlation). Figure 4B shows the mean best-correlation values for each strain resulting from a cross-correlation analysis. These values were significantly higher than the zero-lag correlations (within-strain ANOVAs comparing data from Fig. 4, A and B; P < 0.05). ANOVA of the best-correlation data yielded a significant strain effect (F = 13.20, df = 1,23; P = 0.001) attributable to overall higher correlations for SHR across all days (r = 0.074 higher, on average). There was also a significant day effect (F = 25.40, df = 3,67; P < 0.001) but no strain × day interaction. Post hoc tests indicated that the day effect was attributable to lower correlations on E compared with other days of the cycle (P < 0.001), which did not differ among themselves. The value of the time lag between bins that resulted in the best correlation for each rat was calculated; averages for each strain (Fig. 4C) indicated that ingestive activity in a given bin was best correlated with T_b in a bin ~11 min later. ANOVA of the lag-time measure yielded a significant day effect (F = 4.91, df = 3,67; P < 0.01), and post hoc tests indicated only that the lag time on E was significantly lower than on D2 (P = 0.002).

Fasting and Sucrose Solution Availability in SHR

Figure 5A shows that fasting reduced average dark-phase T_b but that the overall pattern of change in T_b across the cycle was preserved, including an increase in T_b on E. In the ANOVA, T_b data from the separate 2-day fast periods were treated as a single “fasting” ovarian cycle and were compared with the baseline cycle in which food and water were available ad libitum. The analysis yielded a main effect of food availability (F = 91.36, df = 1,54; P < 0.001) and a significant day effect (F = 46.57, df = 3,18; P < 0.001) but no interaction. Post hoc comparisons indicated that T_b was significantly higher on E than on all other days (P < 0.001) and also that T_b was significantly higher on P than on D2, which was the second day without food in the D1-D2 fast. When the second day of each 2-day fasting period was compared (D2 vs. E), T_b was 0.31°C higher on E.

When sucrose solution was available for 2-day periods, total daily energy intake (food kJ + sucrose solution kJ) was significantly elevated above baseline on each day of the cycle, but, unlike the baseline condition shown in Fig. 3A, there was no downturn in energy intake on E. Total caloric intake on D1, D2, P, and E, respectively, was 355.9, 386.6, 384.2, and 358.9 kJ; compared with baseline intake, these values were elevated by 43.8, 51.4, 54.6, and 72.5%, respectively. During sucrose exposure, the majority of dietary energy was obtained by drinking sucrose (D1, D2, P, and E, respectively: 59.11, 64.27, 54.97, and 72.20%), and pairwise comparisons indicated that E was significantly higher than all other days in the cycle in this measure (P < 0.01). Apparently, the willingness of female SHR to ingest calories from sucrose solution is enhanced on E.

The effect of 2-day availability of sucrose solution on average dark-phase T_b was measured in only 13 rats because T_b data were lost for six rats in a computer malfunction. The effect, summarized in Fig. 5B, shows that T_b became elevated above baseline on the second day of each 2-day period that sucrose was available. ANOVA carried out as described for the fasting manipulation yielded a main effect of sucrose availability (F = 8.21, df = 1,36; P < 0.02), an effect of day (F = 136.41, df = 3,12; P < 0.001), and a significant sucrose availability × day interaction (P = 4.20, df = 3,12; P < 0.02). pairwise comparisons indicated that T_b was significantly elevated above baseline on the second day of each sucrose exposure (P < 0.01) and that in both the baseline and sucrose ovarian cycles, T_b was significantly higher on E than on any other day in the cycle (P < 0.001).
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The 2-day fasts resulted in an additional diestrous day being added to the normal 4-day cycle in a majority of the rats: after a D1-D2 fast, 57.9% of the rats showed this effect; after a P-E fast, 68.4% showed it. All rats returned to normal 4-day ovarian cycles after one lengthened cycle. The location of the fast in the ovarian cycle did not significantly affect cycle regularity ($\chi^2 = 2.25, df = 1$). After the 2-day periods of sucrose availability, no rat showed a change in the normal 4-day ovarian cycle pattern.

**DISCUSSION**

$T_b$ in SHR and SD Rats

Our results reveal several unique features of SHR compared with SD rats in measures of dark-phase $T_b$ during the ovarian cycle: 1) average $T_b$ was higher in SHR on E; 2) on D1, D2, and P, $T_b$ in SHR was more variable, and the amplitude of the sawtooth pattern was greater (analysis of standard deviation of dark-phase $T_b$; analysis of high and low $T_b$); 3) in SHR, the low-$T_b$ value increased across days of the cycle and was highest on E, whereas low $T_b$ remained unchanged in SD rats; 4) the high-$T_b$ values were elevated in SHR compared with SD rats on all days of the cycle; and 5) the best correlation between ingestive behavior and $T_b$ resulting from the cross-correlation analysis was significantly higher in SHR than in SD rats. Our finding that the strains did not differ in average dark-phase $T_b$ on D1, D2, or P is in agreement with an earlier finding that chronic $T_b$ in male SHR is not elevated relative to normotensive strains when measurements are made by telemetry (22). The prior evidence for chronically elevated $T_b$ in SHR was from an experiment in which the rectal-probe method was used to measure $T_b$ (6), which involves handling-stress, to which SHR may have been more thermally reactive (22). On the other hand, our findings that, compared with SD rats, SHR had elevated $T_b$ on E, elevated high-$T_b$ values on all days, greater variability in $T_b$ on nonestrous days, and higher best correlations with ingestive behavior, all suggest that SHR have greater thermal reactivity to physiological changes associated with estrus and to behavioral activity required for food and water intake.

In contrast, our findings with SD rats indicated that E had no effect on $T_b$ in either the light or dark phase and that it did not affect variation (sawtooth pattern) in $T_b$. In general, these findings differ from the only prior study in which female SD rats were tested in comparable cage conditions (18). Specifically, on E, we found no significant increase in average $T_b$ in the light phase (the prior study reported $-0.15^\circ C$ elevation relative to D1) or dark phase (the prior study reported $-0.08^\circ C$ elevation relative to D1), and the prominent sawtooth pattern we observed in $T_b$ on D1, D2, and P did not become dampened on E (statistical analyses of both the variation in dark-phase $T_b$ and the high and low-$T_b$ values in each dark phase). The characterization of the sawtooth pattern in the prior report relied on inspection of individual animal records that were 20 times less detailed than ours (10-min bins vs. 30-s bins), and the authors noted that the pattern was most prominent on P and did not occur consistently in every animal. There is no obvious explanation for all the differences between our $T_b$ data and those obtained in the earlier study with SD rats. Both experiments used the same nominal strain of rats and tested them in apparently similar cage conditions ("sedentary" condition in the earlier experiment). We note, however, that the SD rats used in the two studies came from different suppliers of research animals. The strain differences found here highlight the importance of Gordon’s call for more comparative investigations of circadian temperature rhythms in rodents (15).

**Feeding During the Ovarian Cycle**

There was little evidence that the strains differed in ingestive behavior across the ovarian cycle, except that SD rats drank significantly more water than SHR on all days of the cycle. In both strains, food and water intake were reduced on E, as has been reported in many other experiments with various strains (5, 9, 11, 29, 31), and the quantitative properties of ingestive bouts were similar in SD rats and SHR across the ovarian cycle. Although we included both eating and drinking activity in our definition of an ingestive bout, the general pattern of change in bouts defined this way was similar to that reported in some other studies where bouts were defined with respect to feeding activity only (9): there was increased bout frequency, decreased bout duration, and decreased interbout interval on E. The present data on ingestive behavior of SHR during the ovarian cycle are the first to be reported for this strain.

**Effects of Nutritional Manipulations**

Previous studies have shown that $T_b$ and metabolic rate decrease in short-term periods of fasting (26, 33, 35) and that metabolic rate increases in short-term periods of sucrose availability (26). When we fasted SHR for 2-day periods at different points in the ovarian cycle, we found equivalent reductions in $T_b$ from baseline on all days of the cycle. Interestingly, when fasting was initiated on P, absolute $T_b$ actually increased during the second day of fasting, rather than decreasing further as was found when the fast began on D1. Thus, when the second days of fasting were compared, increased estrogen levels on E presumably had thermogenic effects that resulted in dark-phase $T_b$ being $\sim 0.3^\circ C$ higher than was the case on D2 (13). Short-term fasting is known to disrupt the ovarian cycle in hamsters (24) but with differential effects depending on the point in the cycle at which fasting occurs (21). We found delayed ovulation in 56 and 68% of SHR when, respectively, the 2-day fast was initiated on D1 and P. In hamsters, there was no disruption of cycling when the fast began on P (21).
An additional new finding of our work is that, in SHR at least, the availability of sucrose solution prevents the reduction in caloric intake normally observed on E. When 2-day sucrose availability was initiated on D1 or P, T_b always became elevated on E relative to other days in SHR. This is consistent with an elevation in thermogenesis and metabolic rate reported earlier during sucrose availability (26). In contrast to fasting, 2-day periods of increased caloric intake had no effect on cycle length in SHR. In an earlier report, female Wistar rats eating a high-calorie (cafeteria) diet had a longer diestrous phase (14), but that result was obtained after 6 wk on the high-calorie diet. The present data indicate that a short-term decrease in fuel availability (fasting) acts more potently on reproductive status in SHR than a short-term increase in fuels (sucrose availability).

**Relationship Between Ingestive Behavior and T_b**

A main finding of our experiments is that ingestive behavior and T_b are related on all days of the ovarian cycle in SHR and SD rats, but the strength of that relationship is reduced on E compared with D1, D2, and P. Evidence came from analyses of correlations between the duration of ingestive behavior and the value of T_b in 30-s bins of the dark phase. The phase was determined in correlations calculated with zero lag time between the bins and also in cross-correlations where the bins were lagged by different time periods. The cross-correlation analysis revealed significantly higher correlations when the value of T_b in the correlated pairs was from a 30-s bin occurring ~11 min after the bin in which ingestive behavior occurred. Because this time lag yielded the best correlation in both strains, it provides an estimate of the thermal constant of female rats of this size: that is, the thermal consequences of ingestive activity are most fully expressed ~11 min after the activity has occurred. Although a small part of this time lag may be related to the time constant of the temperature-sensitive transmitter (1.4 min; see METHODS), the majority of the lag is determined by the structure and composition of the body.

A speculative possibility is that the correlation between ingestive behavior and T_b is blunt on E because hormonal changes related to estrus provide an added source of thermogenesis on E (13, 20), perhaps including the thermal consequences of increased locomotor activity (9, 18). However, there was evidence of greater thermogenesis in the dark phase of E only for SHR. Our dietary manipulations with that strain supported the idea that factors other than ingestive behavior are responsible for elevating dark-phase T_b on E, at least in SHR: when eating was completely prevented, T_b still became elevated on E relative to other days in the same nutrition cycle. Because hormonal status and general locomotor activity were not measured or manipulated in the present experiment, their possible roles in the present results remain to be investigated.

Future studies of the relationship between ingestive behavior and T_b would benefit from measuring or manipulating hormonal status in rats of different strains and from including measures of general locomotor activity to parse out its contribution to T_b. If hormonal changes associated with E have thermogenic effects that reduce the correlation between ingestive behavior and T_b, direct manipulation of hormonal status in ovariectomized rats of different strains would provide the data necessary to address that question.

Overall, we found that T_b, ingestive behavior, metabolic fuel availability, and ovarian cycling are interrelated in complex ways in female rats of hypertensive and normotensive strains.

We gratefully acknowledge the cooperation and advice of Dr. J. C. Smith and of R. Henderson and P. Hendrick of the Technical Support Group in the Florida State University (FSU) Program in Neuroscience. A. M. Ackert was supported by a National Institutes of Health Joint Neuroscience Predoctoral Training Grant. Some of the data in this study was included in a Master’s thesis submitted by A. M. Ackert to the graduate school of FSU (Spring 2000).

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