The rate of living in tau mutant Syrian hamsters
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Heart rate and body temperature in *tau* mutant Syrian hamsters

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The circadian mutation *tau* shortens the free-running period (τ) of locomotor activity by 4 h in homozygous mutant Syrian hamsters. It also accelerates the resting and the average metabolic rate. The increase in the circadian frequency and in the metabolic rate can be expected to be accompanied by an increase in heartbeat frequency. We tested this prediction in the three genotypes of hamsters (wild-type, τ ≈ 24 h; heterozygous, τ ≈ 22 h, and homozygous *tau* mutants; τ ≈ 20 h). We recorded heart rate during the rest and the active phase of the circadian cycle, and the daily average by simultaneous measurements of heart rate, body temperature, locomotor activity, and metabolic rate at three ambient temperatures (25, 15, and 5°C). The results showed that heart rate and body temperature were similar among the genotypes at rest, activity, and daily average, with a strong effect of ambient temperature. The amplitude of heart rate and body temperature rhythms was indistinguishable between genotypes and dependent on ambient temperature for the body temperature rhythm but not for the heart rate rhythm. Mass-specific oxygen consumption at rest and daily average were only weakly correlated with heart rate. A stronger correlation was found for the active phase.
Introduction

The *tau* mutation in the Syrian hamster shortens the period of the free running rhythms of locomotor activity (Ralph and Menaker, 1988) and body temperature (Refinetti and Menaker, 1992b; Refinetti, 1996). The acceleration of circadian frequency in *tau* mutant Syrian hamsters coincides with an increase in the daily and resting energy expenditure (Oklejewicz et al., 1997, 2000). Homozygous *tau* mutant hamsters with a circa 20% shorter circadian period have an approximately 20% higher energy expenditure for their mass compared with wild-type hamsters. Both of these effects lend interest to a study of the heart rate in *tau* mutant hamsters. The increased metabolic rate might be supported by an increase in heart rate. The same prediction might be made from a general effect of the *tau* mutation on the rate of physiological processes.

Heartbeats occur with a frequency of up to several hundred beats per minute. This periodicity is generated by pacemaker located in a sinus node of the heart and modulated by sympathetic and parasympathetic nervous system (Bozler, 1943). Heart rate and body temperature are characterized by a daily oscillation, they are high in the active phase and low during the rest phase. Thermoregulation and cardiovascular functions, among other behavioral and physiological circadian rhythms, are controlled by the suprachiasmatic nuclei of the hypothalamus, the SCN (Ralph et al., 1990; Refinetti and Menaker, 1992b). Lesions of the SCN abolish or disrupt the circadian pattern of body temperature and heart rate (Warren et al., 1994, Refinetti et al., 1994; Eastman et al., 1984; Saleh and Winget, 1977).

Refinetti and Menaker (1992b, 1993) reported that heart rate in anesthetized animals and body temperature in freely moving hamsters are indistinguishable between homozygous mutant and wild-types. This study further investigates heart rate, body temperature and the corresponding metabolic rate by simultaneous measurements of these parameters in freely moving hamsters (homozygous *tau* mutants with a circadian period of about 20 h, heterozygous mutant with circa 22 h and wild-type hamsters with circa 24 h). We test whether the reduction in the circadian period in locomotor activity is accompanied by increases in the heart rate and body temperature. We performed these tests at three different ambient temperatures. The frequency of heartbeats and body temperature at rest and activity are compared among genotypes, and their relationship to metabolic rate assessed.
Material and methods

Animals and housing conditions

18 six-month-old, male Syrian hamsters (Mesocricetus auratus), 6 of each genotype, were derived from our breeding colony at the Zoological Laboratory. The three genotypes of hamsters, wild-type (\(tau^+/-\)), heterozygote mutant (\(tau^+/-\)), and homozygote mutant (\(tau^-/-\)) were all bred from heterozygous parents (F2 generation). The circadian phenotype was determined after weaning (circa 30 days) by monitoring wheel-running activity for 10 days under constant dim red light. Hamsters were housed in individual cages (l x w x h: 40 x 25 x 15 cm) in a temperature-controlled room (23±0.5 °C) and a 12:12 hours light-dark cycle (LD 12:12) prior to the experiment. Food and water were available ad libitum.

Implantation of transponders

Heart rate transponders (Mini-Mitter Co. Sunriver, OR, model PDT 4000 HR E-mitter-mass 4g) were implanted in the abdominal cavity under sodium pentobarbital anesthesia (150 mg/kg). Two electrode lead wires were attached to the subcutaneous muscle tissue of the chest at a 45-60 degree angle relative to the transverse plane of the heart. Thus, the positive lead was placed on the anterior abdomen wall to the left of the xiphoid process and posterior to the last rib. The negative lead was placed on the right side of chest in the axillary region. The animal cages were placed on a top of energizer/receivers (Mini-Mitter Co., model ER-4000). The number of R-wave pulses of the QRS complex of the cardiac contraction cycle per minute was reported as the heart rate in beats per minute (bpm) by the Vital View acquisition system (Mini-Mitter Co.). Three parameters, heart rate (beats per minute, bpm), body temperature (±0.1 °C), and locomotor activity (arbitrary units) were recorded simultaneously every minute.

Metabolism measurements

For metabolism measurements hamsters were transferred to airtight metabolic cages (l x w x h: 35 x 25 x 25cm) where oxygen consumption (VO2) and carbon dioxide production (VCO2) was measured (see Oklejewicz et al., 1997) simultaneously with HR, Tb and activity. VO2 measurements were conducted in an eight-channel open flow system. Oxygen and carbon dioxide concentration of dried inlet and outlet air (drier: molecular sieves 3 Å, Merck) from each chamber was measured with a paramagnetic oxygen analyzer (Servomex Xentra 4100) and carbon dioxide by an infrared gas analyzer (Servomex 1440). The system recorded the differentials in oxygen and carbon dioxide between dried reference air and dried air from the metabolic cages. Flow rate of the inlet air was
measured with a mass-flow controller (Type 5850 Brooks). Data were collected every 6 min and automatically stored by a computer, which was synchronized to the Vital View PC-system.

**Experiment 1**

Ten days were allowed for recovery after transponder implantation. Then hamsters were transferred to a climate controlled room with constant temperature and constant dim illumination (white light tubes, TLD 36W/85, Philips; illumination range: 5 – 15 lux). There heart rate (HR), body temperature (Tb) and locomotor activity were recorded at three ambient temperatures: 25°C, 15°C and then at 5°C, each for about 72 h after 12 h of acclimatization.

**Experiment 2**

After the last trial at 5°C, hamsters were transferred to the metabolic cages and HR, Tb, locomotor activity were measured simultaneously with records of oxygen consumption and carbon dioxide production. The recordings were conducted at the same ambient temperatures as in the experiment 1 albeit in inverse sequence, first at 5°C, then at 15°C and at 25°C. The trials at each ambient temperature lasted for about 72 h separated by 12 h of acclimatization. Hamsters were weighed before and after each trial to the nearest 0.1 g.

**Data analysis**

All measured variables were expressed in three different ways: (1) the average HR, Tb, VO₂ were calculated as the mean values over the last 48-h of measurement, (2) the minimum HR, Tb, VO₂ were calculated as the lowest value in a 1-h running mean during the rest phase (as indicated by activity counts), (3) the maximum HR, Tb, VO₂ were calculated as the highest value in a 1-h running mean during the active phase (see example in figure 5.1). These three parameters were calculated for each of 18 hamsters at each ambient temperature in both experiments 1 and 2.

The circadian period of HR, Tb and activity counts was determined by Lomb-Scargle time series analysis (Ruf, 1999) at each ambient temperature in experiment 1. Significant differences between the three genotypes and the three ambient temperatures were tested by repeated measure two-way analysis of variance (RM ANOVA; Sokal and Rohlf, 1995). The Pearson product-moment correlation coefficient was computed to test linear correlations between HR and mass-specific VO₂. All results are presented as mean ± standard error unless mention otherwise. Significance was accepted at p<0.05 (two-tailed).
Results

Circadian period of HR, Tb and activity

The circadian period of heart rate (HR), body temperature (Tb) and general locomotor activity simultaneously recorded at 25, 15 and 5°C were independent of ambient temperature (RM one-way ANOVA, p>0.6). Therefore, for each individual circadian periods over the three ambient temperatures were averaged and presented in table 5.1. The variation in circadian periods among HR, Tb, and activity were not significant in any of the three genotypes (RM one-way ANOVA; p>0.1).

Figure 5.1 Representative example of simultaneous measurements of body temperature, heart rate, oxygen consumption and activity for wild-type (tau +/+ ) and homozygous tau mutant (tau -/- ) hamster at 15°C.
The maximum, minimum and average HR and T_b did not significantly differ between the experiments 1 and 2 (paired t-test, p>0.05). The mean values for each ambient temperature are presented in table 5.2. Genotype did not contribute significantly to the explained variance in HR during the resting phase, the active phase, or for the average values at all ambient temperature (RM ANOVA; p>0.5). However, ambient temperature had a profound effect on HR for all calculated parameters (RM ANOVA, p<0.001). Decrease in ambient temperature increased the resting, the active, and the average HR. The increase with the change in ambient temperature from 25°C to 15°C was about twice as large as with the change from 15°C to 5°C (table 5.2). This increase in HR with

<table>
<thead>
<tr>
<th>genotype</th>
<th>HR (bpm)</th>
<th>Tb (°C)</th>
<th>activity</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>tau +/-</td>
<td>24.0 (0.2)</td>
<td>24.2 (0.3)</td>
<td>24.4 (0.3)</td>
<td>0.6</td>
</tr>
<tr>
<td>tau +/−</td>
<td>22.4 (0.5)</td>
<td>22.1 (0.7)</td>
<td>22.4 (0.3)</td>
<td>0.5</td>
</tr>
<tr>
<td>tau −/−</td>
<td>20.7 (0.3)</td>
<td>20.0 (0.4)</td>
<td>20.5 (0.2)</td>
<td>0.3</td>
</tr>
</tbody>
</table>

p differences among rhythms assessed by ANOVA

**HR in the three genotypes of hamsters**

The maximum, minimum and average HR and T_b did not significantly differ between the experiments 1 and 2 (paired t-test, p>0.05). The mean values for each ambient temperature are presented in table 5.2. Genotype did not contribute significantly to the explained variance in HR during the resting phase, the active phase, or for the average values at all ambient temperature (RM ANOVA; p>0.5). However, ambient temperature had a profound effect on HR for all calculated parameters (RM ANOVA, p<0.001). Decrease in ambient temperature increased the resting, the active, and the average HR. The increase with the change in ambient temperature from 25°C to 15°C was about twice as large as with the change from 15°C to 5°C (table 5.2). This increase in HR with

**Table 5.2** Mean values (± S.E.M.) of heart rate (HR) and body temperature (T_b) during the resting phase, active phase and the average over 48-h for the wild type (tau +/-), heterozygous (tau +/−) and homozygous (tau −/−) tau mutant at three ambient temperatures (T_a).

<table>
<thead>
<tr>
<th>T_a</th>
<th>Genotype</th>
<th>Resting phase</th>
<th>Active phase</th>
<th>48-h average</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>T_b (°C)</td>
<td>HR (bpm)</td>
<td>T_b (°C)</td>
</tr>
<tr>
<td>25°C</td>
<td>tau +/-</td>
<td>35.6 (0.2)</td>
<td>264 (7)</td>
<td>37.4 (0.2)</td>
</tr>
<tr>
<td></td>
<td>tau +/−</td>
<td>36.0 (0.1)</td>
<td>267 (6)</td>
<td>37.1 (0.2)</td>
</tr>
<tr>
<td></td>
<td>tau −/−</td>
<td>36.2 (0.3)</td>
<td>264 (3)</td>
<td>37.1 (0.4)</td>
</tr>
<tr>
<td>15°C</td>
<td>tau +/-</td>
<td>35.5 (0.2)</td>
<td>348 (9)</td>
<td>37.2 (0.2)</td>
</tr>
<tr>
<td></td>
<td>tau +/−</td>
<td>35.7 (0.2)</td>
<td>342 (10)</td>
<td>37.1 (0.2)</td>
</tr>
<tr>
<td></td>
<td>tau −/−</td>
<td>35.4 (0.3)</td>
<td>341 (13)</td>
<td>37.1 (0.2)</td>
</tr>
<tr>
<td>5°C</td>
<td>tau +/-</td>
<td>35.1 (0.1)</td>
<td>386 (9)</td>
<td>37.2 (0.1)</td>
</tr>
<tr>
<td></td>
<td>tau +/−</td>
<td>35.2 (0.3)</td>
<td>376 (6)</td>
<td>37.3 (0.1)</td>
</tr>
<tr>
<td></td>
<td>tau −/−</td>
<td>35.3 (0.4)</td>
<td>370 (13)</td>
<td>37.0 (0.3)</td>
</tr>
</tbody>
</table>
decreasing ambient temperature was independent of genotype (interaction of genotype and ambient temperature: p > 0.1; RM ANOVA).

Besides the effect of ambient temperature on HR, we tested whether body mass contributes to the explained variance in HR. Hamsters in this study had the same differences in body weight as reported earlier (Oklejewicz et al., 2001c), although not significantly

Figure 5.2 Relationship of heart rate and metabolic rate of three genotype of hamsters: wild-type (tau +/+), heterozygous (tau +/-) and homozygous tau mutants (tau -/-) at 25, 15 and 5°C. (A) during the resting phase, (B) during the maximal activity, (C) the daily average metabolic rate and heart rate. Linear regressions were fitted for every ambient temperature for the three genotypes pooled together.
so due to the smaller numbers of animals (p=0.06). Tau -/- hamsters had the smallest body weight (average of 118.5 ±4.1g) compared with tau +/- and tau +/+ hamsters (124.6 ±5.8g and 134.3 ±2.5g, respectively). Genotype did not contribute significantly to the explained variance in resting, active, or average HR in the presence of body mass and ambient temperature as explanatory variables (p>0.05).

**Tb in the three genotypes of hamsters**

The resting, active and the daily average body temperature did not significantly differ between genotypes (RM ANOVA, p>0.1). Resting and daily mean Tb decreased with lower ambient temperature (table 5.2). On average the decrease by 10°C in ambient temperature caused a decrease in Tb by 0.38 ±0.09°C. The active Tb was 37.2°C at all ambient temperatures and independent of genotype. Body mass as additional explanatory variable did not significantly add to the explained variance among genotypes in resting, active, and average Tb (p>0.3).

**The amplitude of HR and Tb rhythms**

The amplitude of HR (HR\text{max} – HR\text{min}) was neither dependent on genotype (RM ANOVA, p=0.1) nor on ambient temperature (p=0.1). The average amplitude of HR for all genotypes was 101 ±4 bpm. The circadian amplitude of Tb did also not vary significantly among genotypes (RM ANOVA, p=0.5). The amplitude of Tb, in contrast to HR, was dependent on ambient temperature (p<0.001). The amplitude of Tb increased with decreasing ambient temperature: 1.3 ±0.1°C, 1.5 ±0.1°C and 2.0 ±0.1°C at 25, 15 and 5°C respectively.

**The relationship between metabolic rate and HR**

The mass-specific resting metabolic rate and HR (both calculated over the same 30 min during the rest phase) correlated significantly only for tau +/+ hamsters at 5°C (table 5.3). When the data for the three genotypes were pooled together, the correlation was significant overall at 15°C (correlation coefficient: r=0.53, p=0.03) but not at 25°C and 5°C (p>0.1; figure 5.2A). In the active phase, the maximum VO\textsubscript{2} and heart rate were associated significantly for tau +/- at all ambient temperatures (table 5.3). For all genotypes pooled together, the maximum VO\textsubscript{2} was significantly correlated with HR during the active phase at three ambient temperature (p<0.02; figure 5.2B). The average oxygen consumption (mean over 48 h) positively correlated with the average heart rate for two genotypes (table 5.3). Overall, at 25°C the correlation was close to significance (r=0.43, p=0.08), similar at 15°C (r=0.47, p=0.055) and it just reached significance at
5°C (r=0.47, p=0.0498) for all genotypes pooled together (figure 5.2C).

We tested whether genotype contributed significantly to the explained variance in HR in a model with ambient temperature and mass-specific metabolic rate as explanatory variables. As expected, the active, resting, and daily HR all were significantly dependent on ambient temperatures whereas the genotype did not contribute significantly to the explained variance. The metabolic rate was positively correlated with HR in the active phase, but not significantly so as overall average.

**Discussion**

The circadian period of simultaneously measured heart rate and body temperature was accelerated by the tau mutation in the same way as described for locomotor activity (Ralph and Menaker, 1988). Our data show that the circadian periods of body temperature, heart rate and general activity were statistically indistinguishable and independent of ambient temperature. This is consistent with the small, if any effect of ambient temperature on circadian frequency (Rawson, 1960). The amplitude of the body temperature rhythm and its mean level is affected by locomotor activity, especially in the presence of running wheels (Gander et al., 1986; Refinetti and Menaker, 1992b; 1993; Golombek et al., 1993). In our study lower ambient temperature increased the amplitude of the
body temperature rhythm but not of the circadian rhythm in HR. This was the consequence of a reduction in the resting body temperature.

\textit{tau} mutant hamsters did not show a systematic difference compared with wild-types in their heartbeat frequency, whether measured at rest, activity or daily average. This confirms with the conclusion derived from heart rate recordings of anesthetized \textit{tau} mutant and wild-type hamsters (Refinetti and Menaker, 1993). The \textit{tau} mutation has been shown to affect the ultradian rhythm of hormonal secretion (Loudon \textit{et al.}, 1994) and feeding cycle (Oklejewicz \textit{et al.}, 2001b), but has apparently no effect on the frequency of heartbeats in spite of the difference in metabolic rate (Oklejewicz \textit{et al.}, 1997, 2000). While the circadian pacemaker controls the daily oscillation of heart rate it evidently does not affect the ultradian frequency of cardiac contractions.

The 20\% difference in circadian period and metabolic rate between wild type and homozygous \textit{tau} mutant hamsters is not accompanied by a 20\% difference in the mean heart rate. We recently reported that homozygous \textit{tau} males live on average about 15\% longer than wild-type hamsters in freerunning conditions (Oklejewicz and Daan, 2001e). These data allows us to estimates the number of heartbeats per lifetime. Wild-types, as a consequence of the shorter life, would have on average about 2.75\times10^8 heartbeats per lifetime compared with about 3.10\times10^8 heartbeats in homozygous mutants. Both fall in the lower end of the range (2.1 – 43\times10^8; mean 12\times10^8) of heartbeats per lifetime in mammals (Livingstone and Kuehn, 1979). These figures are only estimates and whether the outcome for wild-types and homozygous mutant hamsters is due to chance can not be tested. Heart rates if anything were slower in homozygous mutants that in wild-types, though not significantly so. In the framework of the ‘rate of living’ theory (Pearl, 1928) such a difference would be consistent with the longer life of mutant hamsters. For the time being it is more important to conclude that the \textit{tau} mutation known and detected because of its accelerating effect on the circadian system certainly does not accelerate heart rate. This is not due to anesthesia involved in Refinetti and Menaker (1993) study, but is true for the resting phase and the activity phase of normal moving hamsters with the gene studied among siblings with similar genetic background.

\textbf{Acknowledgment}

We would like to thank Peter Meerlo and Bauke Buwalda for sharing their expertise in the transmitter implantation procedure. The experiment was conducted under license DEC\#2117/4, University of Groningen.