Daily variations in the influence of noradrenaline on preferred ambient temperature of the Siberian hamster

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Abstract

Daily variations in sensitivity to noradrenaline (NA) and the activation of nonshivering thermogenesis (NST) are important for survival under a potentially wide range of environmental conditions. However, little is known regarding the ability of the Siberian hamster and other species to activate NST in the day and night when they may be subjected to marked variations in environmental temperature. In this study, the effects of acclimation temperature and time of day on the behavioral thermoregulatory response to NA injections in Siberian hamsters (Phodopus sungorus) was investigated. Hamsters were acclimated for 4 weeks to 23°C and a L:D 12:12 h photoperiod. After acclimation, preferred ambient temperatures (PT) in saline- and NA-injected animals were measured continuously in the temperature gradient system. NA (0.6 mg/kg; s.c.) was given every 4 h while PT was monitored. After NA injections there was a rapid drop in PT, decreasing to ~15°C within 10–20 min after each NA injection. Following 4 weeks of acclimation to 10°C and a L:D 8:16 h photoperiod, the same hamsters were re-tested in the temperature gradient system. Cold acclimation led to an accentuation in the behavioral response with a decrease in PT of ~10°C. The maximal decrease in preferred ambient temperatures was recorded during the light phase of the day and during the second part of the night. Lowering of PT after NA allows for rapid dissipation of the heat from NST. Overall, the behavioral response reflects the daily changes in brown adipose tissue sensitivity to NA and thus capacity for NST.

Keywords: Behavioral thermoregulation; Cold acclimation; Daily rhythm; Nonshivering thermogenesis; Noradrenaline; Photoperiod; Preferred ambient temperature; Siberian hamster; Thermal gradient system

1. Introduction

Nonshivering thermogenesis (NST) is a primary source of heat for small mammals when they are exposed to cold and/or during arousal from hibernation and torpor (Smalley and Dryer, 1963; Hayward and Lyman, 1967; Smalley and Dryer, 1967; Jansky, 1973; Heldmaier and Buchberger, 1985). The most important site for NST is brown adipose tissue (BAT). It is localized mainly in the interscapular region. The innervation and vascularization of this tissue are very profound and enables for an extremely high thermogenic capacity (Rauch and Hayward, 1969; Saarela et al., 1989; Cinti, 2001). Noradrenaline (NA), released from the sympathetic nerve terminals, binds to the adrenergic receptors and initiates the sequence of reactions leading to heat generation (Cannon and Nedergaard, 1998). Thermogenic capacity of BAT is dependent on species, ability to enter hibernation or torpor, photoperiodic and thermal history of

Siberian hamsters possess well-developed brown fat that can account for up to 5% of the body mass (Heldmaier and Buchberger, 1985; Heldmaier and Seidl, 1985). Thermogenic effectiveness of BAT is increased by cold exposure and acclimation to short photoperiod (Heldmaier et al., 1982; Heldmaier and Buchberger, 1985; Heldmaier et al., 1985, 1989; Wiesinger et al., 1989; Heldmaier et al., 1990; Wiesinger et al., 1990; Mercer et al., 1994).

Under laboratory conditions, injection or infusion of NA can activate NST (Janský, 1973). When NA-injected animals can behaviorally thermoregulate, they prefer lower ambient temperatures in order to dissipate the excessive heat (Tegowska and Jefimow, 1995; Tegowska et al., 1995a). Degree of lowering of the thermopreferendum after NA injection is correlated with BAT efficiency and the intensity of heat production by means of NST, i.e. preference of lower ambient temperature means higher heat production. Hence, proper thermopreferendum facilitates the maintenance of a constant body temperature but it also requires the precise temperature perception and integration of information from the whole body and environment.

Despite numerous studies on NST only few concern daily variations in its capacity (Kronfeld et al., 1994; Haim et al., 1995a; Haim and Zisapel, 1999; Jefimow et al., 2000). Animals are subjected to large daily and seasonal changes in environmental temperature. Furthermore, mammals exhibit daily rhythms of body temperature and preferred ambient temperature (Refinetti and Menaker, 1992; Gordon, 1994; Refinetti, 1995; Tegowska et al., 1995b; Jefimow and Tegowska, 1996; Refinetti, 1996, 1998a,b). All of these factors influence thermal demands of animals and thus can affect the capacity for NST.

Since thermogenic response to NA, measured as changes in deep body temperature, may vary within the day, it is likely that preferred ambient temperature after NA injection would be also affected by the time of the day. Current experiments were undertaken to examine the effect of the time of the day as well as acclimation to cold and short photoperiod on the influence of NA on preferred ambient temperature of Siberian hamsters. This species lives in a severe climate of continental Asia (Weiner, 1987b) and experiences marked daily and seasonal differences in environmental temperature. During night time activity they are exposed to cooler ambient temperatures and it would activate BAT thermogenesis (NST). Furthermore, environmental temperature is much lower during winter. Thus, thermogenic capacity of BAT in animals acclimated to cold and short photoperiod (winter conditions) should be higher than in non-acclimated ones. Taken together, daily and seasonal variations in NST capacity would allow for proper adjustment to environmental changes.

2. Materials and methods

Six adult male Siberian hamsters (Phodopus sungorus) were used. Animals were bred, raised and kept in the laboratory under 12:12 h light:dark schedule (L:D 12:12), at an ambient temperature ($T_a$) of 21 ± 2°C. Animals were housed individually in standard laboratory cages with food and water available ad libitum. Prior to cold acclimation, animals were maintained on L:D 12:12 with lights on at 09:00 h, at an $T_a$ of 23 ± 2°C. After 4 weeks body weight was measured (mean body weight 29.75 ± 1.07 g) and each animal was tested individually in the thermal gradient system (non-acclimated group). Next, animals were transferred to a cold environment (10°C) and short photoperiod (L:D 8:16, lights on at 09:00 h) for 4 weeks. They were weighed again (mean body weight 30.10 ± 1.20 g) and experiments in the thermal gradient system were repeated (acclimated group).

The experiments on preferred ambient temperature were conducted in a thermal gradient system. This system consists of long aluminium chamber (120 cm length, 10 cm height, 8 cm width) divided, by half-width partition, into 16 compartments of the same size, and covered with transparent Perspex to permit light entry. The system is heated at one end and cooled at the other end, resulting in a range of temperatures increasing linearly from 5 to 45°C. The position of animal (and thus selected temperatures) was detected in 1-s intervals by infrared photoemitter–photodetector pairs placed in each compartment, and saved on disk.

Each animal was placed in thermal gradient system for 3 days. On the first day of experiment, hamster was undisturbed, that enabled exploration...
of the new environment. On the next day, starting from the beginning of the light phase (09:00 h), NA ((±)-Arterenol, Sigma) at a dose of 0.6 mg/kg or saline (in the same volume as NA) was injected subcutaneously (s.c.) every 4 h. It has been shown that this dose of NA induces maximal stimulation of NST in this species (Böckler et al., 1982). Night time injections were made under dim red light. Half of the animals was injected firstly with NA (2nd day) and then with saline (3rd day). The sequence of injections in the latter half was opposite (1st day-undisturbed, 2nd day-saline, 3rd day-NA). In both groups (non-acclimated and acclimated) experimental procedure was the same, preferred ambient temperature (PT) after saline and NA injections was measured continuously in daily cycle. Food and water were present in the thermal gradient throughout the experiment.

All recorded data were plotted in 10 min intervals and they are plotted as mean±S.E. To compare the daily mean of PT, the data collected for 1 h after each injection were discarded. In each hamster daily mean of PT, mean PT's during day and night were calculated and Student’s t-test was used for comparison. Data collected 30 min before and 90 min after each injection were used for statistical analysis (Student’s t-test between corresponding 10-min intervals) to assess effects of saline and NA on the thermal preferendum. Three-way ANOVA with post hoc test (LSD) was used to investigate the influence of acclimation, time of day, and time following the injection (10-min intervals) on the difference in thermopreferendum of saline- and NA-injected hamsters.

In the Section 1 and Section 4 we use ‘Siberian’ and ‘Djungarian’ interchangeably in spite of that they are different species (Ross, 1995, 1998). We preserved original nomenclature used by cited authors. Nevertheless, all papers discussed here concern Phodopus sungorus (Siberian hamster).

3. Results

3.1. Daily mean of preferred ambient temperature

In non-acclimated hamsters PT was similar in saline- and NA-injected animals. There was also no day–night difference in PT. In hamsters acclimated to cold and SP PT was lower during the night than during the day. In saline-injected animals this difference was 1.2 °C (P < 0.01) and in NA-injected ones 1.8 °C (P < 0.05). In acclimated

<table>
<thead>
<tr>
<th></th>
<th>Non-acclimated</th>
<th>Acclimated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Saline</td>
<td>NA</td>
</tr>
<tr>
<td>Total</td>
<td>29.3 ± 0.7</td>
<td>28.1 ± 0.8</td>
</tr>
<tr>
<td>Day</td>
<td>29.4 ± 0.7</td>
<td>28.1 ± 0.8</td>
</tr>
<tr>
<td>Night</td>
<td>29.3 ± 0.8</td>
<td>28.1 ± 0.9</td>
</tr>
</tbody>
</table>

Significantly different values are indicated by the superscripts: a and b, P < 0.01; c and d, P < 0.05.

animals injected with NA PT during the light phase of the day was higher than in non-acclimated hamsters (P < 0.05). The sequence of injections did not influence changes in PT. Detailed data on PT are presented in Table 1.

3.2. The influence of acclimation, time following the injection and time of day on the response to NA

NA injections caused marked lowering of thermopreferendum lasting up to 40 min (Figs. 1 and 2). Acclimation to cold and SP led to an accentuation in this behavioral response to NA (3-way ANOVA: F(1, 339) = 9.35; P < 0.01). In non-acclimated hamsters the lowest PT after NA injection was 17.0 ± 2.2 °C (injection at 13:00 h) and it was lower by 12.2 °C than in saline-injected animals (P < 0.01). The largest difference in PT of saline- and NA-injected hamsters (12.9 °C, P < 0.02) was recorded after the injection at 05:00 h. In acclimated hamsters these differences were much more pronounced. The minimal PT’s were recorded after NA injection at 09:00 and 05:00 h (10.8 ± 1.6 and 10.9 ± 2.6 °C, respectively). The differences between PT’s of saline- and NA-injected animals were also larger than in non-acclimated hamsters. Detailed data on PT after each injection of NA are presented in Table 2.

The influence of time following the NA injection on the degree of the PT lowering was very significant (3-way ANOVA: F(5, 339) = 43.5; P < 0.001). The lowest selected temperature was observed 10–20 min following the injections except for the injection at 01:00 h in non-acclimated hamsters. At that time it occurred earlier, i.e. during the first 10 min.
Fig. 1. Mean preferred ambient temperature of non-acclimated Siberian hamsters (N=6). Dark bars indicate dark phase of the day, and arrows indicate the times of injections. Panel (a): time course of mean $PT_a$ (± S.E.) in saline-injected (black line) and NA-injected (grey line) animals. Areas surrounded with the rectangles are enlarged in panel (b). Panel (b): mean $PT_a$ (± S.E.) 30 min before and 90 min after each injection. Each point is the average of the previous 10 min. Open circles—NA, full circles—saline. Asterisks indicate significant difference between $PT_a$ of saline and NA-injected hamsters: *$P<0.05$, **$P<0.01$, ***$P<0.001$.

Time of the day significantly influenced the response to NA (3 way ANOVA: $F(5, 339)=2.45$; $P<0.04$).

As determined by post hoc analysis (at $\alpha=0.05$) the drop in $PT_a$ after the injection at 13:00 h was markedly lower than after the injections at
Fig. 2. Mean preferred ambient temperature of Siberian hamsters acclimated to cold and SP (N = 6). Dark bars indicate dark phase of the day, and arrows indicate the times of injections. Panel (a): time course of mean $PT_r$ (± S.E.) in saline-injected (black line) and NA-injected (grey line) animals. Areas surrounded with the rectangles are enlarged in panel (b). Panel (b): mean $PT_r$ (± S.E.) 30 min before and 90 min after each injection. Each point is the average of the previous 10 min. Open circles—NA, full circles—saline. Asterisks indicate significant difference between $PT_r$ of saline and NA-injected hamsters: *$P$ < 0.05, **$P$ < 0.01, ***$P$ < 0.001.

09:00 ($P$ < 0.02), 17:00 ($P$ < 0.03) and 01:00 h ($P$ < 0.05). $PT_r$ recorded after NA injection at 05:00 h was also lower than after the injections at 09:00 ($P$ < 0.02) and 17:00 h ($P$ < 0.05).

4. Discussion

Behavioral thermoregulation is very important for small mammals since it is less energetically
Table 2
Minimum preferred ambient temperatures in NA-injected hamsters (within 10–20 min following the injection except for the injection at 13:00 h in non-acclimated hamsters when minimum was recorded during the first 10 min) and the difference from saline-injected animals

<table>
<thead>
<tr>
<th>Time</th>
<th>Absolute (°C)</th>
<th>Difference from saline</th>
<th>Non-acclimated</th>
<th>P</th>
<th>Acclimated</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Non-acclimated</td>
<td>Acclimated</td>
<td>Non-acclimated</td>
<td></td>
<td>Acclimated</td>
<td></td>
</tr>
<tr>
<td>09:00</td>
<td>18.5±1.5</td>
<td>10.8±1.6</td>
<td>8.8</td>
<td>0.01</td>
<td>17.8</td>
<td>0.01</td>
</tr>
<tr>
<td>13:00</td>
<td>17.0±2.2</td>
<td>13.7±2.1</td>
<td>12.2</td>
<td>0.01</td>
<td>14.8</td>
<td>0.001</td>
</tr>
<tr>
<td>17:00</td>
<td>18.1±1.7</td>
<td>14.8±2.4</td>
<td>9.3</td>
<td>0.01</td>
<td>15.1</td>
<td>0.01</td>
</tr>
<tr>
<td>21:00</td>
<td>17.6±1.6</td>
<td>12.4±2.7</td>
<td>11.9</td>
<td>0.001</td>
<td>16.5</td>
<td>0.001</td>
</tr>
<tr>
<td>01:00</td>
<td>18.9±1.5</td>
<td>13.7±2.7</td>
<td>11.6</td>
<td>0.01</td>
<td>14.6</td>
<td>0.01</td>
</tr>
<tr>
<td>05:00</td>
<td>18.7±3.3</td>
<td>10.9±2.6</td>
<td>12.9</td>
<td>0.02</td>
<td>19.3</td>
<td>0.001</td>
</tr>
</tbody>
</table>

costly than autonomic responses. Proper ambient temperature allows achieving thermal homeostasis without launching autonomic mechanisms, i.e. it requires relatively little energy. In current experiments we did not measure body temperature nor oxygen consumption and the magnitude of reaction to NA (i.e. NST capacity) was defined on the behavioral basis, i.e. changes in thermopreferendum.

The behavioral thermoregulatory response to select cooler ambient temperatures in response to NA injection reflects the thermogenic sensitivity of BAT to NA. Overall, acclimation to cold and short photoperiod leads to a marked behavioral sensitivity to NA. That is, the behavioral thermoregulatory response in the cold acclimated hamsters to NA was nearly double that of the non-acclimated animals. Interestingly, the behavioral response to NA was affected by time of day with more profound responses occurring in the middle of the day and at the end of night. Since night time would be associated with activity and exposure to colder ambient temperatures, the slight reduction in behavioral sensitivity to NA during the first part of night may reflect the fact that BAT thermogenesis is already activated at night. Further stimulation with NA is not as effective as is seen during the day time.

4.1. Daily mean of PT<sub>a</sub>

In many species PT<sub>a</sub> varies within a day, i.e. it is 180° out of phase with body temperature rhythm and activity (Gordon, 1994; Tęgowska et al., 1995b; Refinetti, 1995; Jefimow and Tęgowska, 1996; Refinetti, 1996, 1998a,b). Siberian hamsters are nocturnal rodents and exhibit higher body temperature at night. We recorded that in acclimated hamsters PT<sub>a</sub> was lower during night than during day, by approximately 1.2 °C in saline-injected hamsters and by approximately 1.8 °C in NA-injected ones.

One might expect that acclimation to cold and SP would induce changes in PT<sub>a</sub>. However, we did not record changes in daily mean of PT<sub>a</sub> due to acclimation. It can be suggested that 4 weeks of acclimation (that we applied in this study) is too short period to induce changes in PT<sub>a</sub>, or PT<sub>a</sub> does not undergo seasonal variations. Longer acclimation is also needed to elicit morphological changes, such as decrease in body weight and molt to a winter pelage (Hoffmann, 1973; Heldmaier and Steinlechner, 1981). Undoubtedly, all examined hamsters were responding to SP. Starting from 8th week of acclimation changes in fur coloration and body weight as well as daily torpor were observed (unpublished observation). In this study, the only significant effect of acclimation on PT<sub>a</sub> was recorded in hamsters receiving NA during the light phase of the day, i.e. it was higher in acclimated animals. It could be a consequence of more pronounced drop in PT<sub>a</sub> after NA injections. In previous work we found that NA induced short increase in deep body temperature followed by marked decrease (Jefimow et al., 2000). Thus, hamsters could move to the warmer part of the thermal gradient to keep thermal balance and prevent further hypothermia that was previously observed in hamsters that had no possibility of temperature selection.

4.2. Effect of acclimation on the behavioral response to NA

Acclimation or acclimatization of the Siberian hamsters to SP and cold induces increase in NST
capacity (Heldmaier and Buchberger, 1985; Heldmaier et al., 1985; Heldmaier and Lynch, 1986; Heldmaier et al., 1989; Wiesinger et al., 1989; Heldmaier et al., 1990; Wiesinger et al., 1990; Mercer et al., 1994). Heldmaier et al. (1990) demonstrated that limit of cold tolerance was markedly lowered during winter, e.g. from −24 to −70 °C in Djungarian hamsters. The increase in cold tolerance was due to enlarged capacity for NST. 66.2% of all NST in cold-adapted hamsters is derived from BAT while in warm-adapted animals only 37% (Heldmaier and Buchberger, 1985).

The increase in BAT efficiency during cold season is especially important for small mammals like Siberian hamsters. They live in severe climate of continental Asia and stay active throughout the year. Winter is very cold and the snow cover is almost absent (Weiner, 1987a). Low ambient temperatures are often encountered in winter when animals are leaving their burrows and nests. Heat generation by NST is energetically more efficient and comfortable compared to increase in maximal metabolic rate or shivering thermogenesis (Kuroshima, 1993).

In current experiments, NA injections elicited cold-seeking behavior. It indicates that body temperature was elevated without changes in set-point (Gordon, 1993). Due to thermogenic action of NA, hamsters were moving to the colder part of the thermal gradient in order to dissipate the excessive heat. In this way temperature gradient between animal and environment was enlarged what facilitated heat loss. When calorigenic action of NA was coming to end, hamsters returned to warmer ambient temperatures that were similar to temperatures preferred before the injection and to temperatures preferred by saline-injected animals. Acclimation to cold and SP led to an accentuation of PT

\[ T \]

right after the injection at 09:00 h. On the other hand, the smallest drop in PT

\[ T \]

right after the injection at 09:00 h and at the beginning of the night (17:00 h). One might suggest that repeated injections in the same animals could not reflect an effect of time of day. However, we always compared the influence of NA with the influence of saline at the same time so if repeated stress of injection could affect response to NA then it would be also present in saline-injected animals. Furthermore, before successive injections hamsters were returning to temperatures preferred by saline-injected animals (Figs. 1 and 2). We can also exclude adaptation of adrenergic receptors since there was no gradual attenuation (or augmentation) of the response to NA.

Similar daily rhythm of the response to NA was recorded in golden spiny mice (Kronfeld et al., 1994) and common spiny mice (Haim and Zisapel, 1999). Minimal effects were observed at the beginning and end of the dark phase while maximal responses were recorded in the middle of the dark and the light phase of the daily cycle, i.e. when body temperature and oxygen consumption of control animals had the lowest values. As suggested by authors, it could be a result of changes in the level of endogenous NA occupying adrenergic receptors and we cannot exclude it. Not only availability but also sensitivity of adrenergic receptors could contribute to observed rhythmicity. Opposite results were obtained by Haim et al. (1995a). In wood mice, the minimal response to NA was recorded in the late afternoon, i.e. during the lowest value of body temperature and maximal response when body temperature was high. Such variations were explained by changes in GDP-
binding. Redlin et al. (1992) found that GDP-binding to BAT mitochondria (that reflects thermogenic activity of BAT) in juvenile rats was lower during the low phase of body temperature rhythm than during the high phase (Redlin et al., 1992). Therefore, effectiveness of exogenous NA might be lower when body temperature is kept at the low level.

Daily variations in thermoregulatory responses cannot be excluded since thermoregulation is relaxed during the resting (inactive) phase of the day (Repinetti and Menaker, 1992). Watts and Repinetti (1996) also suggested that sensitivity of autonomic thermoregulatory system to cold underwent circadian variations. The aim of our study was to examine behavioral response to NA. On the basis of obtained results we have no doubt that this response exhibits daily rhythm, although we are not able to give univocal explanation of its cellular and physiological background.

4.4. The significance of daily rhythmicity of the response to NA

The question is whether observed rhythmicity of the influence of NA on PTa is only a direct result of daily variations at the cellular and molecular level or it has more important, e.g. ecological meaning?

The different pattern of the response to NA in wood mice on the one hand (Haim et al., 1995a), and golden spiny mice (Kronfeld et al., 1994) and common spiny mice (Haim and Zisapel, 1999), on the other hand, might be a result of different habitats of these animals. Golden and common spiny mice are desert rodents, living in hot and arid environment and encountering great fluctuations in ambient temperature. Wood mice were captured in Scotland where climate is rather temperate. Siberian hamsters inhabit arid steppes and semi-arid deserts of continental Asia (Weiner, 1987b). The pattern of the response to NA that we recorded in Siberian hamsters resembles that observed in golden and common spiny mice. Thus, there are some similarities in the habitats of hamsters and mice that could induce similar thermoregulatory responses. It is likely that Siberian hamsters can use NST for short time thermoregulation as suggested for golden spiny mice (Kronfeld et al., 1994). Because of relatively high metabolic rate (Heldmaier and Steinlechner, 1981; McNab, 1983) they would save energy when possible, particularly as they live in cold and severe climate. Short time thermoregulation allows rapid elevation in body temperature when it is necessary. During winter hamsters leave their burrows several times to search for food, and at that time they are exposed to severe cold. The episodes of activity last from several minutes to approximately 2 h (Ruf and Heldmaier, 2000). Exposure to cooler ambient temperatures during activity would activate BAT thermogenesis (NST). Thus, endogenous NA would occupy adrenergic receptors and further stimulation with exogenous NA is not as effective as during the day. A marked behavioral sensitivity to NA that we recorded during the second part of the night (05:00 h) does not contradict this hypothesis since sleep–wake cycle of Siberian hamsters is not unimodal. During light phase of the day they spend 67% of time asleep and 43% during the dark phase (Deboer et al., 1994).

Much more pronounced decrease in PTa of acclimated hamsters after NA injections, indicates and confirms the role of NST in seasonal aclimatization. Undoubtedly, BAT efficiency and thus NST capacity in Siberian hamsters exhibits daily rhythm. This rhythm seems to be correlated with activity pattern and environmental conditions. It would allow for proper adjustment to the environment and facilitates the maintenance of a thermal homeostasis and energy balance.

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