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DAILY VARIATIONS IN THE RESPONSE OF WOOD MICE *APODEMUS SYLVATICUS* TO NORADRENALINE

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Summary

Non-shivering thermogenesis (NST) is an important mechanism for heat production in many small rodent species. Daily rhythms of body (rectal) temperature (T_b) reflect the relationship between heat production and heat dissipation. The roles of photoperiod and the time of day at which NST is measured were studied in wood mice *Apodemus sylvaticus*.

Mice of both sexes (N=18) were acclimated to two different photoperiod regimes (16 h:8 h L:D and 8 h:16 h L:D) at a constant ambient temperature (T_a) of 24 °C. Non-shivering thermogenesis was measured as the maximal response of oxygen consumption ($\dot{V}_{O_2NA}$) and body temperature (T_{bNA}) to a noradrenaline (NA) injection (1.5 mg kg^{-1} subcutaneously) in unanaesthetized mice at three different times in the daily rhythm of T_b (N=6 in each group).

Mice acclimated to 8 h:16 h L:D had a greater response to noradrenaline at the three different times of the day compared with those acclimated to 16 h:8 h L:D. The extent of the response to noradrenaline within each group varied with time of day; the smallest response was recorded at 18:00±1.5 h, and the greatest was at 01:00 h. These results suggest that photoperiod is an important cue for seasonal acclimatization in this species and that the response to noradrenaline follows a daily rhythm.

Key words: thermoregulation, photoperiod, non-shivering thermogenesis, brown adipose tissue, daily rhythms, wood mouse, *Apodemus sylvaticus*.

Introduction

To thermoregulate effectively, small mammals such as rodents depend on heat production by non-shivering thermogenesis (NST) when exposed to cold (Jansky, 1973). Acclimation to cold or to long scotophase (short photoperiod) increases NST capacity upon subsequent exposure to low ambient temperatures (Lynch, 1970; Heldmaier, 1972; Haim and Fourie, 1980; Heldmaier *et al.* 1981). The thermoregulatory significance of NST was summarized by Jansky (1973), and the ecological significance of this important mechanism for heat production in rodents was recently emphasized by Haim and Izhaki (1993).

NST is commonly estimated by measuring the maximal oxygen consumption ($\dot{V}_{O_2NA}$) in response to a subcutanecus noradrenaline injection, using a dose per body mass as calculated by Heldmaier (1971). As the measurements of NST are carried out at 1 °C below the lower critical point ($\dot{V}_{O_2min}$), it was suggested that NST capacity should be calculated as the ratio between $\dot{V}_{O_2NA}$ and $\dot{V}_{O_2min}$ (Borut *et al.* 1978). However, Klaus *et al.* (1988) refer to NST capacity as the difference between these two values; that is $\dot{V}_{O_2NA}$ minus $\dot{V}_{O_2min}$.

The daily rhythm of body temperature (T_b) in small rodents is well documented (Aschoff, 1982; Heldmaier *et al.* 1989). From these studies, it has been established that, during the resting period, T_b can be up to 2 °C below the values measured during the active phase. As the daily rhythm of T_b reflects two different daily rhythms, that of heat production and that of heat dissipation, it may be assumed that the drop in T_b during the inactive period is partly a result of a decrease in heat production due to the reduction in resting metabolic rate (RMR) (Haim *et al.* 1988). Does NST capacity show a daily rhythmicity and does photoperiod have any effect on such rhythms?

The aim of this study was to measure NST at different hours of the day in wood mice *Apodemus sylvaticus*, in order to establish whether a daily rhythm exists. As the mice were acclimated to two different photoperiod regimes, but were kept at a fixed temperature, it was possible to assess the impact of this environmental cue on this rhythm.

Materials and methods

The mice (N=18; males and females) were captured 10 km north of Aberdeen, Scotland (57 °N), in March 1993. All mice

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were acclimated for at least 3 weeks to each of two different photoperiod regimes in series (16 h:8 h L:D and 8 h:16 h L:D) at a constant ambient temperature (T_a) of 24±1 °C. Each mouse was kept in a cage with a sawdust substratum, woodwool bedding and a small cylindrical tube, which was used as a nesting place. The mice were fed *ad libitum* on rodent chow and water. Fresh carrots were added to the diet twice a week. Food was available until the beginning of NST measurements. A dim red light was kept on constantly in order to facilitate handling of the mice during the dark period. During the light phase, a bright light was left on continuously. Prior to NST measurements, the daily rhythm of T_b was established in each group (Haim *et al.* 1994). Non-shivering thermogenesis (NST) was measured, under both conditions of acclimation, at three different times of the day: 'noon' (11:00–13:00 h), late afternoon (17:00–19:00 h) and 'midnight' (00:30–03:00 h).

Non-shivering thermogenesis was measured both as the maximal oxygen consumption response ($\dot{V}_{O_2NA}$) (calculated as the mean maximal value recorded over a period of 10 min) and as the body temperature response (T_{bNA}) of unanaesthetised mice to a subcutaneous noradrenaline injection of 1.5 mg kg^{-1} (Heldmaier, 1971). For this purpose, the mice were moved into a metabolic chamber (volume 600 ml), which was kept inside a temperature-controlled incubator (Gallenkamp 1NL-0101N). The lights inside the incubator were adjusted to the photoperiod regime to which the mice were acclimated. The T_a at which the measurements were carried out was 1 °C below the lower critical temperature (Haim *et al.* 1994): 28 °C for long-photophase-acclimated (LP-mice) and 26 °C for long-scotophase-acclimated (LS-mice). Oxygen consumption was measured using an open-flow system (Depocas and Hart, 1957; Hill, 1972). Dried air was pumped through the metabolic chamber at a rate of 480 ml min^{-1} using a Wrights DM3A precision cumulative flow (water displacement) meter (Alexander Wrights Ltd, Westminster, England).

Oxygen concentration of the dry excurrent air was determined by an oxygen analyzer (Servomex 1100OH, Servomex Ltd). Oxygen concentration was monitored by recording the O_2 values every 3 min over the measuring period. The mean $\dot{V}_{O_2}$ values obtained over a 30 min period, provided that they did not differ by more than 0.05 %, were recorded as $\dot{V}_{O_2min}$, and the T_b recorded at the end of this stage was taken as $T_{b,min}$. Body (rectal) temperature was measured using a copper–constantan thermocouple connected to a digital thermometer (model 751-K, Digitron Instrumentation Ltd). After establishing $\dot{V}_{O_2min}$ and $T_{b,min}$ values, noradrenaline was injected. $\dot{V}_{O_2}$ measurements went on for about 1 h after noradrenaline injection. T_{bNA} was measured within 15 min after $\dot{V}_{O_2NA}$ had been established. All $\dot{V}_{O_2}$ values were corrected to STPD. As the tested mice were not anaesthetised, they were observed throughout the whole measurement period, and only those periods in which they were inactive were included in the results ($\dot{V}_{O_2NA}$), as suggested by Ellison and Skinner (1990).

All data are given as means ± one standard deviation. The data were analyzed using one-way analysis of variance (ANOVA) (Minitab). The ANOVA was followed by *a posteriori* Scheffe pairwise comparison of means (P<0.05). Significant relationships between the two photoperiod treatments were established by using Student's *t*-test.

Results

The minimum $\dot{V}_{O_2}$ recorded was significantly affected by photoperiod (ANOVA, F=66.5, P<0.001) and time of the daily T_b rhythm at which it was measured (ANOVA, F=8.99, P<0.01). Mice of both groups showed a different response to noradrenaline at different hours of the day. The smallest response in both groups was in the late afternoon. Minimal

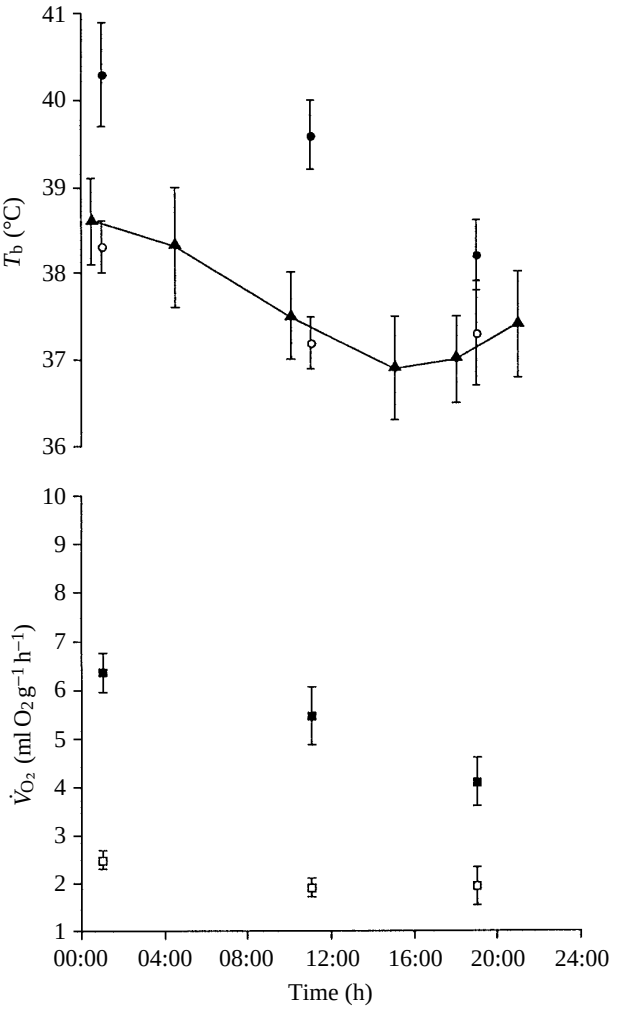

Fig. 1. The response of wood mice *Apodemus sylvaticus* acclimated to a long photophase (16 h:8 h L:D) (LP-mice) to a noradrenaline injection at different hours of the day. Open symbols represent minimal oxygen consumption ($\dot{V}_{O_2}$) and body (rectal) temperature (T_b) measured at an ambient temperature of 28 °C. Filled symbols represent maximal $\dot{V}_{O_2}$ and T_b measured as a response to noradrenaline. All values are mean ± S.D. for six mice at each time in each of the the two different photoperiod regimes. The line joining the triangles describes the daily rhythm of T_b measured in these mice. Values are mean ± S.D., N=9.

values of $\dot{V}_{O_2min}$ and $T_{b,min}$ were also recorded at this time of the day. The $\dot{V}_{O_2min}$ values recorded for LS-mice ($2.60\pm0.4\,ml\,O_2\,g^{-1}\,h^{-1}$; for a body mass of $23.1\pm2.8\,g$; N=6) were significantly (P<0.01) higher than those recorded for LP-mice ($1.95\pm0.4\,ml\,O_2\,g^{-1}\,h^{-1}$; for a body mass of $22.5\pm2.1\,g$; N=6). The response to noradrenaline injection in *A. sylvaticus* was significantly affected both by acclimation to photoperiod (ANOVA, F=104.2, P<0.001) and by the time of day that noradrenaline was administered (ANOVA, F=45.4, P<0.001). The maximal response to noradrenaline ($\dot{V}_{O_2NA}$ and T_{bNA}) was higher in LS-mice, whereas NST capacity ($\dot{V}_{O_2NA}/\dot{V}_{O_2min}$) and $T_{b,min}$ were the same in the late afternoon in both groups (Figs 1 and 2).

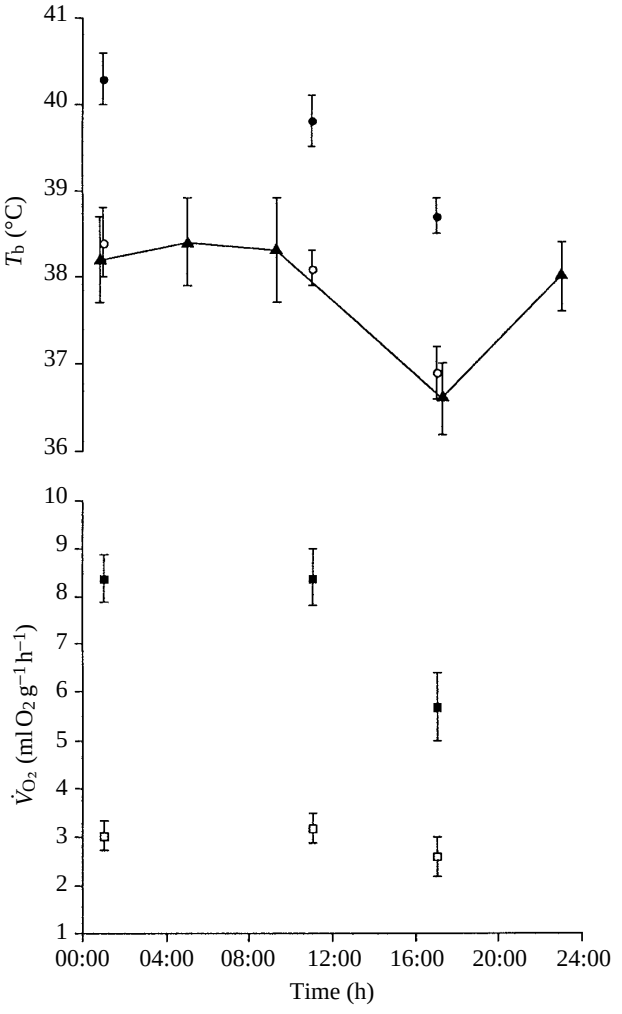

Fig 2. The response of wood mice *Apodemus sylvaticus* acclimated to a long scotophase (LS-mice) (8 h:16 h L:D) to a noradrenaline injection at different hours of the day. Open symbols represent minimal oxygen consumption ($\dot{V}_{O_2}$) and body (rectal) temperature (T_b) measured at an ambient temperature of 26 °C. Filled symbols represent maximal $\dot{V}_{O_2}$ and T_b measured as a response to noradrenaline. All values are mean ± S.D. for six mice at each time in each of the two different photoperiod regimes. The line joining the triangles describes the daily rhythm of T_b measured in these mice. Values are mean ± S.D., N=9.

LP-mice showed the maximal response to noradrenaline at 'midnight', whereas in LS-mice there was no difference between the response obtained at 'midnight' and that at 'noon'. The $\dot{V}_{O_2NA}$ values recorded for LS-mice at 'midnight' and at 'noon' ($8.43\pm0.5\,ml\,O_2\,g^{-1}\,h^{-1}$ and $8.40\pm0.2\,ml\,O_2\,g^{-1}\,h^{-1}$ respectively) were significantly (P<0.01) higher than those recorded for LP-mice ($6.37\pm0.4\,ml\,O_2\,g^{-1}\,h^{-1}$) at 'midnight'. However, T_{bNA} values of LS-mice at 'midnight' and 'noon' were similar to those of LP-mice measured at 'midnight'. As $\dot{V}_{O_2min}$ values of LS-mice at both times ($3.03\pm0.3\,ml\,O_2\,g^{-1}\,h^{-1}$ and $3.20\pm0.3\,ml\,O_2\,g^{-1}\,h^{-1}$ respectively) were higher than those of LP-mice ($2.50\pm0.2\,ml\,O_2\,g^{-1}\,h^{-1}$) measured at 'midnight', NST capacity did not differ between these groups (Figs 1 and 2).

A high NST capacity was obtained in LP-mice measured at 'noon'. In these mice, the $\dot{V}_{O_2}$ response to noradrenaline was not especially high ($5.49\pm0.6\,ml\,O_2\,g^{-1}\,h^{-1}$) but, as the $\dot{V}_{O_2min}$ value was low (only $1.92\pm0.2\,ml\,O_2\,g^{-1}\,h^{-1}$), NST capacity was high. In such mice the difference between T_{bNA} and $T_{b,min}$ was also high (Fig. 1).

The NST capacity values calculated in two different ways ($\dot{V}_{O_2NA}/\dot{V}_{O_2min}$; $\dot{V}_{O_2NA} - \dot{V}_{O_2min}$), as well as ΔT_b values, for the two studied groups at the different times are given in Table 1.

Table 1. *Non-shivering thermogenesis in wood mice* Apodemus sylvaticus *measured at different hours of the day*

Photo-period	Time of NA injection (h)	$\dot{V}_{O_2NA}/\dot{V}_{O_2min}$	$\dot{V}_{O_2NA}-\dot{V}_{O_2min}$ (ml O_2 g^{-1} h^{-1})	ΔT_b (°C)
16L:8D	01:00	2.55 ± 0.1^b	3.87 ± 0.2^a	2.0 ± 0.6^a
LP-mice	12:00	2.90 ± 0.1^a	3.57 ± 0.5^a	2.4 ± 0.6^a
	18:00	2.10 ± 0.2^c	2.17 ± 0.3^b	0.8 ± 0.3^b
		F=29.2;	F=34.3;	F=14.7;
		P<0.0001	P<0.0001	P<0.001
8L:16D	01:00	2.8 ± 0.4^a	5.40 ± 0.6^a	1.9 ± 0.3^a
LS-mice	11:30	$2.7\pm0.2^{a,b}$	5.20 ± 0.5^a	1.7 ± 0.2^a
	17:30	2.10 ± 0.5^b	3.15 ± 0.6^b	1.8 ± 0.5^a
		F=5.2;	F=26.0;	F=0.7;
		P<0.05	P<0.001	P<0.05

$\dot{V}_{O_2NA}/\dot{V}_{O_2min}$, NST measured as the ratio between the maximal oxygen consumption response to noradrenaline ($\dot{V}_{O_2NA}$) and the minimal value ($\dot{V}_{O_2min}$) measured 1 °C below the lower critical point.

$\dot{V}_{O_2NA}-\dot{V}_{O_2min}$, NST measured as the difference between these two values.

ΔT_b, the increase in body temperature as a response to noradrenaline.

All values are mean ± S.D. for six mice at each time in each of the two different photoperiod regimes.

Significantly different values among different times of noradrenaline injection (P<0.05, using Scheffe pairwise comparison of means) are indicated by the superscripts a–c: a>b>c.

NA, noradrenaline; NST, non-shivering thermogenesis; LP-mice, mice acclimated to a long photophase; LS-mice, mice acclimated to a long scotophase.

Discussion

Regulatory non-shivering thermogenesis (NST), which is measured as the response to a noradrenaline injection, is an important mechanism for heat production upon exposure to cold as well as an essential component of cold tolerance in small mammals (Jansky, 1973; Heldmaier *et al.* 1982). The response to a noradrenaline injection was found to be higher in several rodent species that had been acclimated to a long scotophase, even at relatively high ambient temperatures (Lynch, 1970; Haim and Fourie, 1980; Heldmaier *et al.* 1981; Haim, 1982; Ellison *et al.* 1992). As all the wood mice in our study were acclimated to an ambient temperature of 24 °C, the largest response to noradrenaline injection of LS-mice compared with that of LP-mice is probably attributable to acclimation to the different photoperiod regimes. This suggests that regulatory NST is an important mechanism for heat production in *A. sylvaticus* from higher latitudes.

The circadian rhythm of T_b has been measured in many mammalian species. Circadian rhythms of heat production and heat dissipation have also been studied in mammals in relation to body mass (Aschoff, 1982). As heat production shows a daily rhythm, it would be reasonable to predict that regulatory NST, measured as a response to a noradrenaline injection, would also show a daily rhythm. Such a daily rhythm could be the expression of a change in the sensitivity of the brown adipose tissue (BAT) receptors to the neurotransmitter, a daily change in the neurotransmitter secretion, or a combination of both. Recently, Redlin *et al.* (1992) have studied the daily changes of BAT thermogenesis in juvenile rats. As BAT is highly important for thermogenesis in newborn rats, the thermogenic state of BAT was tested during the maximum and minimum phases of the T_b cycle using guanosine 5′-diphosphate (GDP) binding to BAT mitochondria as an index of thermogenic activity. The results of their study showed that a decrease in T_b during the resting phase of the circadian cycle was accompanied by a significant decrease in GDP binding activity.

The results of our study show that the minimal response to noradrenaline in mice from both acclimated groups was in the late afternoon. The minimal T_b values in mice of both groups were also recorded at this time of the daily cycle. As the addition of exogenous noradrenaline did not increase heat production and the $\dot{V}_{O_2min}$ values recorded at this time were low, we suggest that this is consistent with the response of the receptors to low noradrenaline levels. These results are in agreement with those of Redlin *et al.* (1992), in which GDP binding activity was low when T_b was low. The differences in the maximal response to noradrenaline between the two groups were also reflected by the daily rhythm of T_b. Whereas LS-mice had a large response to noradrenaline at 'noon' as well as at 'midnight', LP-mice had a larger response at 'midnight' compared with that at 'noon'. These results fit very well with the daily T_b rhythm. LS-mice at 'noon' still have a high T_b, whereas in LP-mice the value recorded is close to the minimal value measured at 15:00 h. The high values at 'midnight' in both groups are in agreement with the high $T_{b,min}$ and $\dot{V}_{O_2min}$ values recorded at this time. Therefore, we predict that, as in juvenile rats (Redlin *et al.* 1992), GDP binding activity will also be maximal at this point of the daily cycle in these mice.

Calculating NST capacity by using the ratio between $\dot{V}_{O_2NA}$ and $\dot{V}_{O_2min}$ reveals that in LP-mice this variable differed between the three different times of the day. However, when subtracting $\dot{V}_{O_2min}$ from $\dot{V}_{O_2NA}$ to calculate NST capacity, as suggested by Klaus *et al.* (1988), no significant difference was recorded for NST capacity in LP-mice when the results at 'noon' were compared with those at 'midnight'. Even using this method of calculation in these mice, NST capacity was significantly lower in the late afternoon than at 'noon' or at 'midnight' (Table 1).

The present study focuses on the daily rhythms of T_b and NST capacity at different times of the day as a response to changes in photoperiod. No attempt was made to establish the underlying mechanisms. We do not rule out the possibility of changes in activity, feeding behaviour, etc. However, they are outside the aims of the present study. The results show that photoperiod is a cue for seasonal acclimatization of the NST mechanism in *A. sylvaticus* from high latitudes. The physiological and biochemical mechanisms of the response to noradrenaline at the different hours of the day should be studied further.

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