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Cardiorespiratory and metabolic reactions during entrance into torpor in dormice, *Glis glis*

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Summary

Dormice voluntarily enter torpor at ambient temperatures ranging between 0–28°C. This study describes heart rate, ventilation frequency, O_2-consumption (defined as metabolic rate), CO_2-production and body temperature during entrance into torpor. Their temporal relationship was analysed during the time course of metabolic depression at different ambient temperatures. Body temperature and heart rate were measured in unrestrained dormice with implanted transmitter. Ventilation frequency was monitored by total body plethysmography or infrared video monitoring. To compare entries into torpor at different T_a these periods were distinguished into four different phases: the resting phase prior to torpor, the phase of pre-torpor adjustments, the reduction phase and the phase of steady state torpor. In the pre-torpor phase, dormice increased their ventilation, metabolic rate and heart rate, indicating that the torpid state is initiated by an enhanced metabolic activity for about an hour. This was followed by a rapid reduction of ventilation, metabolism and heart rate, which reached their minimum values long before body temperature completed its decline. The results of the present study show that the entrance into torpor is caused by an active respiratory, cardiac and metabolic depression.

Key words: *Glis glis*, torpor entrance, metabolic depression, ventilation, heart rate.

Introduction

Physiological functions during deep torpor and arousals have been repeatedly studied in different species of hibernators (Lyman and Chatfield, 1950; Hammel, 1985; Trachsel et al., 1991; Daan et al., 1991; Fons et al., 1997; Wilz and Heldmaier, 2000). However, our knowledge about entrance into hibernation is rather limited. This is partly due to the fact that entrance into hibernation occurs spontaneously, and can hardly be predicted or even initiated by the experimenter. The use of anaesthetics or other methods to initiate hypothermia may generate transitions into a hypometabolic state that are not comparable to spontaneous entry into torpor. Since body temperature and metabolic functions are reduced during entrance into torpor, it is tempting to assume that metabolic depression is based on temperature effects (Snapp and Heller, 1981; Geiser, 1988; Song et al., 1996, 1997). However, several studies in woodchucks, marmots and squirrels have shown that metabolic rate is reduced much faster than the development of hypothermia, indicating a temperature-independent depression of metabolic rate (Lyman, 1958; Heldmaier et al., 1993; Ortmann and Heldmaier, 2000; Heldmaier et al., 2004). The final depression of metabolic rate is accomplished by a synergistic action of temperature effects and metabolic inhibition (Heldmaier and Elvert, 2004).

The edible dormouse (*Glis glis*) used in this study is known as a true hibernator. They retreat into an underground burrow, their hibernaculum, from October through March/April. However, they may also become torpid during summer months. This may either be short daily torpor or extended periods of estivation (Wilz, 1999). We obtained long-term records of metabolic rate, heart rate, body temperature and ventilation frequency in dormice spontaneously entering and arousing from torpid states throughout the year. On average, each individual was recorded for about five months, which allowed continuous observation of torpor episodes at different ambient temperatures ranging from 0–28°C. The records were used to analyse the sequence of physiological depression during spontaneous entries into torpor. The timely relationship between heart rate, ventilation, metabolic rate and body temperature may reveal whether all these changes occur in parallel, or if they follow different time courses, indicating a hierarchy of physiological inhibitions during entrance into torpor. It will also answer the question whether metabolic rate, ventilation and heart rate depression are a consequence of developing hypothermia or are downregulated separately.

Materials and methods

Animals

The dormice (*Glis glis* L.) used in this study were bred and raised with food and water *ad libitum* under natural photoperiod at Marburg University. Adult males were implanted with temperature and ECG transmitters and adapted to constant short photoperiod (L:D regime 10 h:14 h). Body weight at the beginning of experiments varied between 140 and 180 g. During the experiments the dormice were housed in a wire mesh cage inside a climate chamber (0.8×0.5×0.4 m) under short photoperiod. The sleep and nesting box (volume 2 l) was placed outside the cage, but with free access through a revolving door to the cage (Wilz and Heldmaier, 2000). Temperature inside the climate chamber varied between 0 and 28°C and the humidity was maintained at 80±10%. During measurements of torpor no food was supplied but water supply remained *ad libitum*. Continuous records of body temperature, metabolic rate, heart rate and ventilatory frequency of a single dormouse lasted for periods of up to 10 months while the measurements were carried out from April 1998 to February 2001 with 146 torpor episodes monitored. When body weight dropped below 110 g during the experiments the dormice were refed with sunflower seeds, nuts, rodent chow and apples.

Body temperature and heart rate

Core body temperature (T_b) and heart rate (f_H) were recorded by a modified physiological implantation system (Data Sciences, DSI, St Paul, USA). The temperature-sensitive transmitter (model TA10ETA-F20; DSI, St Paul, USA) was calibrated in a water bath within temperature ranges of 0.5–42°C before implantation. Coefficients were calculated from the regression equation (Elvert and Heldmaier, 2000a). The dormice were anaesthetized with ketamine (60 mg kg^{-1}) and xylacine (4 mg kg^{-1}). Anesthetics were injected i.p. The transmitter for recording T_b, f_H and electrocardiogram (ECG) was implanted into the abdominal cavity and fixed with sewing silk (1.5 metric) to the peritoneum. The electrodes of the transmitter were sutured subcutaneously in the area of the right shoulder and the lower left chest, corresponding to an Einthoven II recording (see Kramer et al., 1993). Peritoneum and skin were sutured with resorbable catgut. Following the operation the dormice were kept for recovery for 3 weeks at 18–20°C with food and water *ad libitum*. A magnetic switch allowed the transmitter to be turned off to prolong battery life and records could be obtained during repeated sessions for up to 2 years in individual dormice.

The receiver (RPC-1, DSI, St Paul, USA) was placed below the sleeping box (Fig. 1) and was connected to a consolidation matrix (DSI, St Paul, USA), which powered the receiver and transmitted the signals to an universal analog adapter (UA10, DSI, St Paul, USA). The adapter was calibrated by transmitter specific coefficients and controlled by a commercial computer software (Chart, DSI, St Paul, USA). The UA10-analog adapter and the computer control unit constitute the telemetry control unit (Fig. 1). For data analysis the signals were interfaced (interface 1: DACpad-71 B, Datalog, Moenchengladbach, Germany) and stored in 1 min interval on computer hard disk. The data acquisition was controlled by a self-developed software (QB45), which also included filter algorithms for suppression of noise and interference. The monitoring of the heart rate was done by scanning the signal input with a sampling rate of 1 kHz for 10 s. When several peaks were detected a mean value of peak interval was calculated. A continuous recording of ECG during entrance into torpor was enabled by splitting the analog output of the UA10-adapter and connecting an additional interface (UIM100A, model MP100, Biopac Systems, Santa Barbara, USA) with a sampling rate of up to 2 kHz. Data were stored on a further computer system.

Metabolic rate

Oxygen consumption (metabolic rate, $\dot{M}_{O_2}$) and carbon dioxide production were recorded by pumping air through the nesting box with a flow rate of about 35 l h^{-1}. The air was dried by cooling traps (M&C Cooler, EPC, Ratingen, Germany). The flow rate was measured by electronic flow rate meters (FM 360, Tylan, Eching, Germany). This post-cuvette flow rate was corrected by the RQ to obtain a pre-cuvette flow rate for calculation of metabolic rate. O_2 and CO_2 content was measured by an O_2-analyzer (Ametek S 3a/II, Pittsburgh, USA) and a CO_2-analyzer (UNOR 6N Maihak, Hamburg, Germany). Both analysers continuously compared the air from the nesting box with reference air from the climate chamber

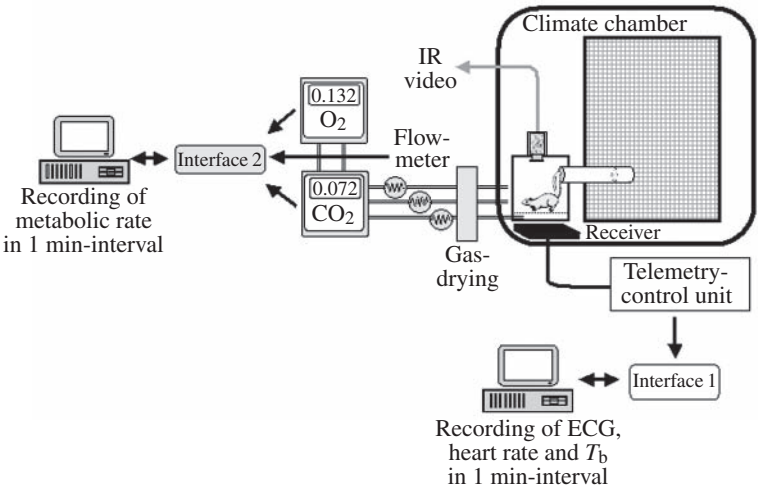

Fig. 1. Experimental setup for measuring body temperature (T_b), heart rate (f_H), electrocardiogram (ECG) and metabolic rate ($\dot{M}_{O_2}$). For measuring ventilation frequency (f_V) the nesting box was replaced by a total body plethysmograph and a differential pressure transducer. On top of the nesting box an infra red camera was fixed for observing the dormice. The receiver was placed below the nesting box or the plethysmograph, respectively. For further information see text.

and provided a resolution of 0.001% for O_2 and CO_2. A magnetic valve system allowed switching to a second reference channel every 55 min for automated zero readjustment and calibration checks for 5 min. $\dot{M}_{O_2}$ (ml O_2 h^{-1}) was calculated according to the equation by Heldmaier and Steinlechner (1981).

$$\dot{M}_{O_2} = (\Delta\text{vol\% } O_2) \times \text{flow rate} \times 10 .$$

Analog outputs of gas analysers were interfaced (interface 2: MDP 8280, Datalog, Moenchengladbach, Germany) and data were stored by a self-developed software (QB45) in 1 min intervals on computer disk.

Ventilation frequency

Two different methods were used for monitoring the ventilation frequency (fV). In a first set of experiments it was measured by video recordings of ventilatory movements with an infrared camera on top of the sleep and nesting box. This allowed measurements of ventilation in resting dormice. In a second experimental design, the nesting box was replaced by a total body plethysmograph to record ventilatory frequency even during transitions, e.g. when entering torpor. The plethysmograph consisted of two chambers with 810 ml of volume each (Malan, 1973). The dormice spontaneously entered one chamber, which was then used for measuring, and the other one served as a reference chamber. Both chambers were closed when the dormice settled quietly in the plethysmograph. A continuous and identical air flow through both chambers supplied air to the dormice and allowed recording of $\dot{M}_{O_2}$ in parallel. Second exits from both chambers were connected to a differential pressure transducer (Halstrup EMA 48, Germany, range ± 50 Pa, accuracy 1%), which continuously compared the air pressure inside the chambers. Pressure fluctuations caused by ventilation of the dormouse (temperature changes of inspired and expired air) were continuously recorded and interfaced by the data acquisition system described above (Biopac Systems, Santa Barbara, USA). Body temperature, fH and ECG were simultaneously recorded by the receiver directly placed below the plethysmograph. Ambient temperature (T_a) was measured with a thermocouple placed inside the sleeping box and the animal chamber of the plethysmograph, respectively. Ventilation frequency was analysed in 5 min intervals with a commercial software package (AcqKnowledge, Version 3.5.3, Biopac Systems). From fV and oxygen consumption we calculated the oxygen pulse to demonstrate the fast response and interaction of fV and $\dot{M}_{O_2}$.

Data analysis

We focussed our interest on monitoring the entrance into torpor at different ambient temperatures. Wilz and Heldmaier (2000) demonstrated in dormice that the classification of dormancy into hibernation, estivation and daily torpor due to seasonal responses is based on the same physiological mechanisms for downregulation. Therefore, we concluded the recorded data as entrances into torpor, regardless of duration of torpor bouts. The acquired data were analysed using SigmaPlot 7.0. Statistical calculations were performed with SigmaStat 2.01, Jandel Scientific. Mean values are reported ± S.D. Data were tested for normality with Kolmogorov-Smirnov and correlation coefficients were calculated following Spearman.

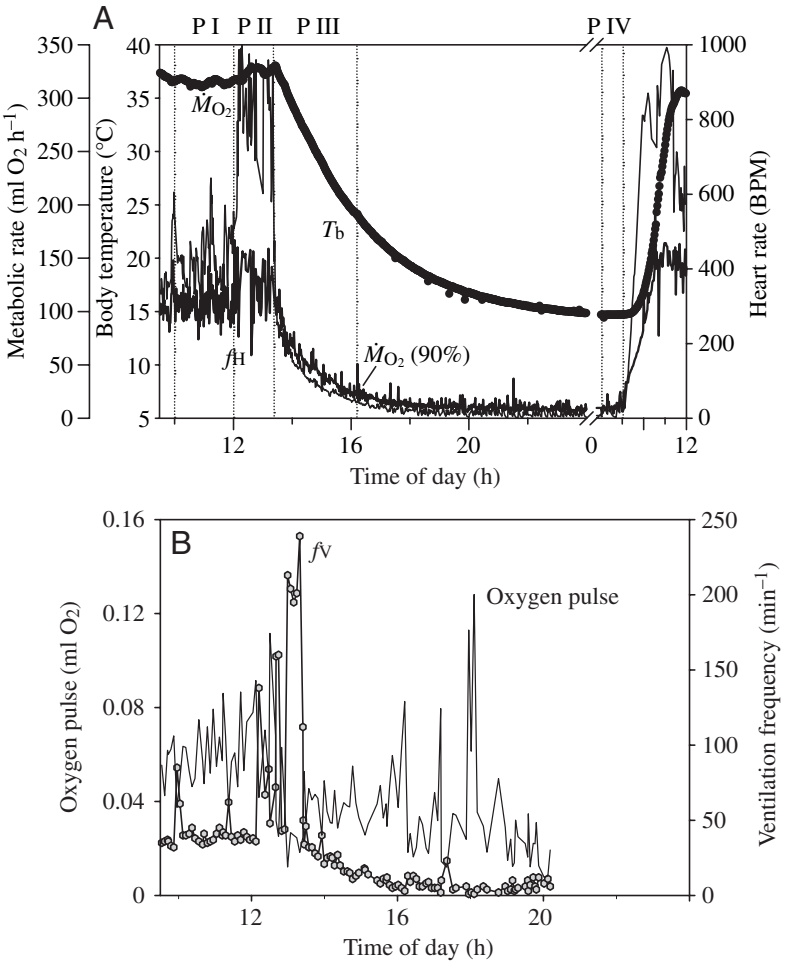

Fig. 2. (A) Entry into torpor in a dormouse (#R4S, BW=120 g) at 15°C T_a. Body temperature (T_b, black filled circles), metabolic rate ($\dot{M}_{O_2}$, solid black line) and heart rate (fH, thick solid black line) were recorded simultaneously. The different phases of entries into torpor are shown in a 4 day sample recording, broken after the first day, from 1–5 August, 2000. Four different phases were defined: phase I (P I) as a resting phase, phase II as the pre-torpor phase, phase III was defined as the period lasting from the initial peak through 90% of transition towards values reached in steady state of torpor. Dotted lines indicate beginning and end of single phases. The end of P III is determined by 90% reduction value of RMR during P I. The 90% decrease time of fV, fH and T_b are calculated separately. Phase IV is defined as the phase of lethargy. (B) The same entry into torpor as in Fig. 2A, but additionally the ventilation frequency (fV, gray circles) and the oxygen pulse for ventilation are shown (compare Fig. 5B). Note the high ventilatory frequency and the simultaneous low oxygen pulse occuring prior to entrance into torpor.

Results

Time course of entrance into torpor

The time course of entrance into torpor always followed the same pattern. To analyse it we discriminated four different phases. Resting values of $\dot{M}_{O_2}$, f_H, f_V and T_b before entrance into torpor are assigned to phase I. Prior to the metabolic depression we observed an increase in $\dot{M}_{O_2}$, f_H and f_V which was assigned to phase II. In most cases it also was associated with slight increases in T_b. A last peak of metabolic rate marked the beginning of metabolic depression and the development of hypothermia, which was defined as phase III, and steady state in torpor is classified as phase IV (Fig. 2).

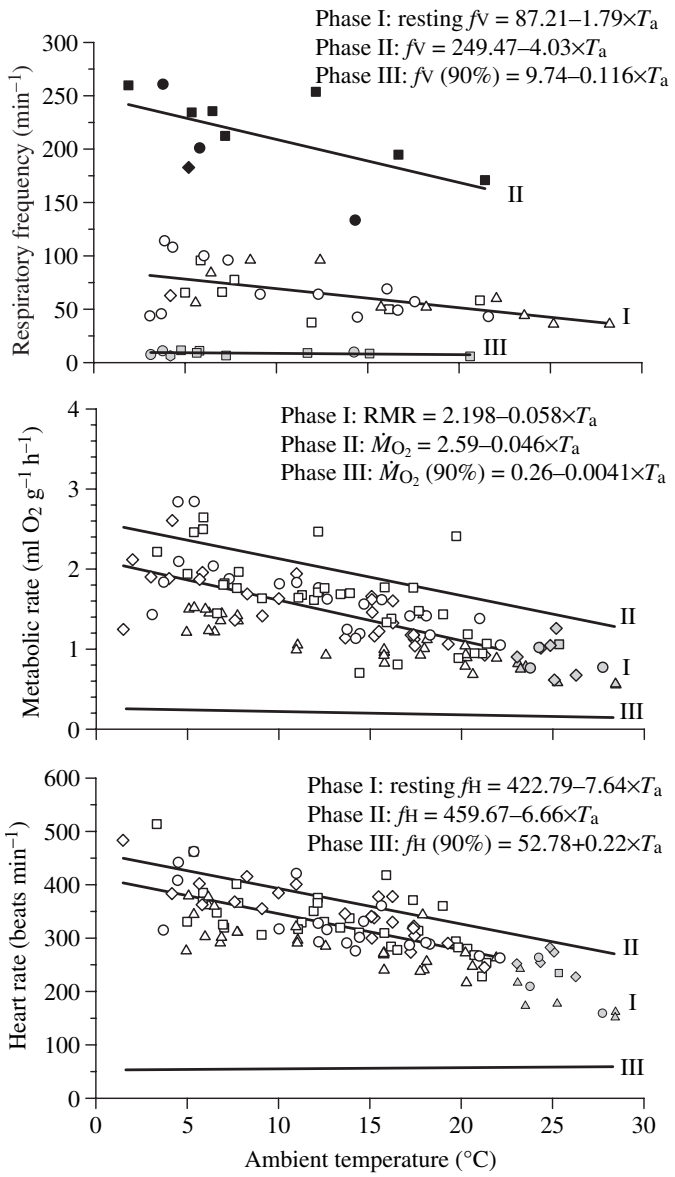

Fig. 3. Effect of T_a on f_V, $\dot{M}_{O_2}$ and f_H during the different phases (I–III). Top: change of f_V, individual dormice are marked with symbols: circles #R4S, squares #L6S, triangles #R13G, diamonds #R3S. Open symbols indicate phase I (N=5, n=31, r^2=0.33, P<0.001), black symbols indicate phase II (N=3, n=11, r^2=0.37, P<0.05), dark grey symbols indicate phase III (N=3, n=11, r^2=0.013, P>0.05); middle: change of $\dot{M}_{O_2}$, light grey symbols in phase I of $\dot{M}_{O_2}$ and f_H (middle and bottom, N=4, n=125, r^2=0.468, P<0.001) indicate values measured in the thermoneutral zone; phase II (N=4, n=146, r^2=0.27, P<0.001), phase III (N=4, n=107, r^2=0.571, P<0.001); bottom: change of f_H, phase I (N=4, n=121, r^2=0.58, P<0.001), phase II (N=4, n=141, r^2=0.567, P<0.001), phase III (N=4, n=105, r^2=0.574, P<0.001).

Phase I – resting values prior to entrance into torpor

Prior to entrance into torpor the dormice rested quietly in their sleeping box, in a hunched posture. Only occasional movements for cleaning their fur could be observed by an IR video camera. Dormice were perfectly normothermic and their T_b remained constant at 36.7±0.79 (N=4, n=107; where N=no. of animals, n=no. events). Oxygen consumption was 0.552±0.051 ml O_2 g^{-1} h^{-1} at 28°C T_a and was elevated to 2.1±0.311 ml O_2 g^{-1} h^{-1} at 2°C T_a (Fig. 3, middle). The mean oxygen consumption per unit body weight of dormice during phase I was 1.42 ml O_2 g^{-1} h^{-1}. Heart rate during phase I was defined as resting heart rate. At a thermoneutral T_a of 28°C resting f_H was maintained at 170–200 beats min^{-1}. In the cold it increased with $\dot{M}_{O_2}$ and reached about 500 beats min^{-1} at 1.5°C T_a (Fig. 3, bottom).

The ventilation pattern during resting phase I was always characterized by regular and quiet breathing movements (Fig. 4). This resting ventilation was about 40 breaths min^{-1} at 28°C T_a. It increased in the cold to 114 breaths min^{-1}, as shown by the record in Fig. 3, top.

Phase II – pre-torpor adjustments

The resting phase was terminated by a sudden increase of $\dot{M}_{O_2}$, f_H and f_V (Fig. 2). The mean duration of this phase was 47 min. Ventilation rate rose to more than 260 breaths min^{-1} at 1.9°C T_a, which is about five times the resting ventilation rate (Fig. 3, top). The high frequency included lower amplitudes of pressure changes and low volumes of ventilation that closely resembles the ventilation pattern of panting. The high frequency alternated with slower and regular breathing periods (Fig. 4). Hyperventilation was accompanied by a decrease in oxygen pulse, determined from oxygen consumption and ventilation rate (sample recordings in Figs 2B and 5B). During the resting phase the mean oxygen pulse was 54 µl O_2 breath^{-1} and decreased to 27.4 µl O_2 breath^{-1} during pre-torpor phase. T_b slightly rose to 36.97±0.91°C. The high ventilation rate was accompanied by a significant increase in $\dot{M}_{O_2}$ up to 80% above resting $\dot{M}_{O_2}$ to 1.99±0.59 ml O_2 g^{-1} h^{-1} (Fig. 3, middle). Mean heart rate increased significantly preceding entrance into torpor from 314.8 beats min^{-1} during resting to 372.3 beats min^{-1} during pre-torpor phase (Figs 2, 3, bottom).

Phase III – Reduction of physiological parameters

The period with high ventilation and $\dot{M}_{O_2}$ was suddenly terminated, and the dormice entered metabolic depression. In most cases this starting point was marked by a peak in breathing frequency and $\dot{M}_{O_2}$. Therefore, the last metabolic peak was chosen as starting point of the reduction phase or

phase III. Since all parameters showed a nonlinear transient decline, which did not allow a precise determination of the end point of transition, we calculated the 90% decrease time instead, using RMR during the resting phase as the initial value and $\dot{M}_{O_2}$ during deep torpor (see phase IV) as the final value. The duration of this decrease time was determined for $\dot{M}_{O_2}$, f_H, f_V and T_b.

At all ambient temperatures the reduction of f_V, $\dot{M}_{O_2}$ and f_H occurred much faster than that of T_b (transition time of f_V *versus* $\dot{M}_{O_2}$ versus f_H, n.s. (P>0.05). The reduction of f_V started with a steep decrease and dropped almost to the level of resting ventilation within a few minutes. At 15°C T_a the f_V decreased from 200–240 breaths min^{-1} during pre-torpor phase to 40–50 breaths min^{-1} during reduction phase (Fig. 2). At 5°C T_a f_V was 300–350 breaths min^{-1} during pre-torpor phase and decreased rapidly to 30–40 breaths min^{-1} (Fig. 5). The calculation of the 90% decrease time, e.g. in this sample recording at 5°C T_a revealed 82 min. It then decreased continuously until the onset of intermittent ventilation (Fig. 4). The same pattern of steep reduction from high frequency into torpor was observed at all other temperatures, too.

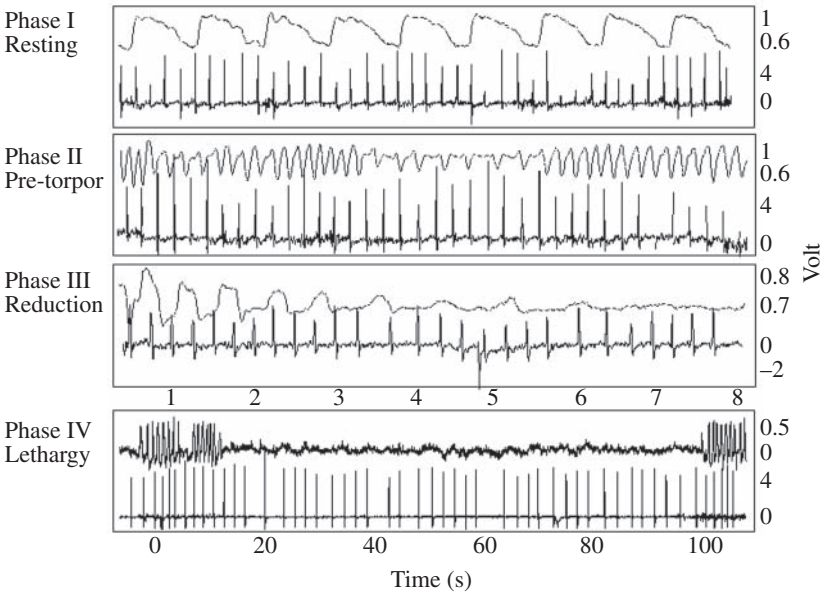

Fig. 4. Monitoring of f_H and change of ventilation during the entrance into torpor in the different phases. While phase I–III have the same time scale, it is enlarged in phase IV, due to the intermittent breathing pattern during deep torpor.

The slope of $\dot{M}_{O_2}$ reduction always paralleled that of f_H (Figs 2, 5). At 5°C T_a the decrease time of $\dot{M}_{O_2}$ required 83 min and f_H 93 min to perform 90% of the transition into torpor. Comparable sample recordings of entrance into torpor at different T_a show that the slope of reduction of $\dot{M}_{O_2}$ for 5 and 15°C T_a is similar (Figs 2, 5). The oxygen pulse recovered again during the beginning of the reduction period, but decreased later to a mean value of 26 µl O_2 breath^{-1} at the end of reduction phase. The slope of the decline of T_b increased at low T_a and was most rapidly at the beginning of entrance into torpor, indicating an exponential time course of T_b. To compare the slopes of T_b reduction the maximum cooling rate was determined between 32–30°C T_b and standardised as cooling rate per hour. At 5°C T_a the cooling rate was at 8.5°C h^{-1} and dropped at 28.2°C T_a to 1.1°C h^{-1}. The 90% decrease time required was 343 min, which is about three times the time required for metabolic, cardiac and ventilation reduction.

Phase IV – Steady state torpor

To obtain stable minimum values for physiological variables in deep hibernation we used the final period of a torpor bout, i.e. 60–20 min prior to spontaneous arousal. T_b during steady state torpor approached minimum values. Minimum $\dot{M}_{O_2}$, f_H and T_b were determined as average values over this period. Due to the small temperature gradient between expired and ambient air it was impossible to determine ventilation during steady state topor.

Metabolic rate was reduced to a fraction of that observed during the resting phase (Fig. 2). Within a temperature range of 7–14°C the mean value of $\dot{M}_{O_2}$ was unchanged at 0.0382±0.0037 ml O_2 g^{-1} h^{-1}, showing no relation with ambient temperature and, thus, with body temperature (r^2=0.097, P>0.05, N=26). At T_b below 7°C the $\dot{M}_{O_2}$ of individual dormice increased again and they showed regulatory heat production preventing a further decrease of T_b. The onset of this regulation varied individually. The minimal $\dot{M}_{O_2}$ observed was 0.0174 ml O_2 g^{-1} h^{-1} at T_b of 4.8°C for dormouse #R13G. At a T_b of 14°C the minimal $\dot{M}_{O_2}$ was 0.038 ml O_2 g^{-1} h^{-1} and increased to 0.25 ml O_2 g^{-1} h^{-1} at 28°C T_b (Fig. 6).

Heart rate in steady state torpor was only a fraction of that observed during resting (e.g. at 7°C T_b 8.2 beats min^{-1} instead of 314.8 beats min^{-1} in the awake but resting state). Below 7°C T_b the f_H accelerated corresponding to the elevated $\dot{M}_{O_2}$ to defend minimum T_b (Fig. 6). Above 7°C T_b the f_H in torpid dormice increased, depending on the temperature up to 66 beats min^{-1} at 31°C T_b (Fig. 6).

Discussion

The present study demonstrates that entrance into torpor is achieved by a timed schedule of depression of metabolic rate, heart rate and ventilation frequency from normothermia to deep torpor. They are reduced in parallel and appear to be closely linked to each other. This reduction always occurs in the same coordinated manner during entrance into hibernation, estivation or daily torpor and is only little affected by temperature. We therefore analyse the time course and investigate the interaction of different simultaneously measured parameters to supplement the knowledge of mechanisms of metabolic depression.

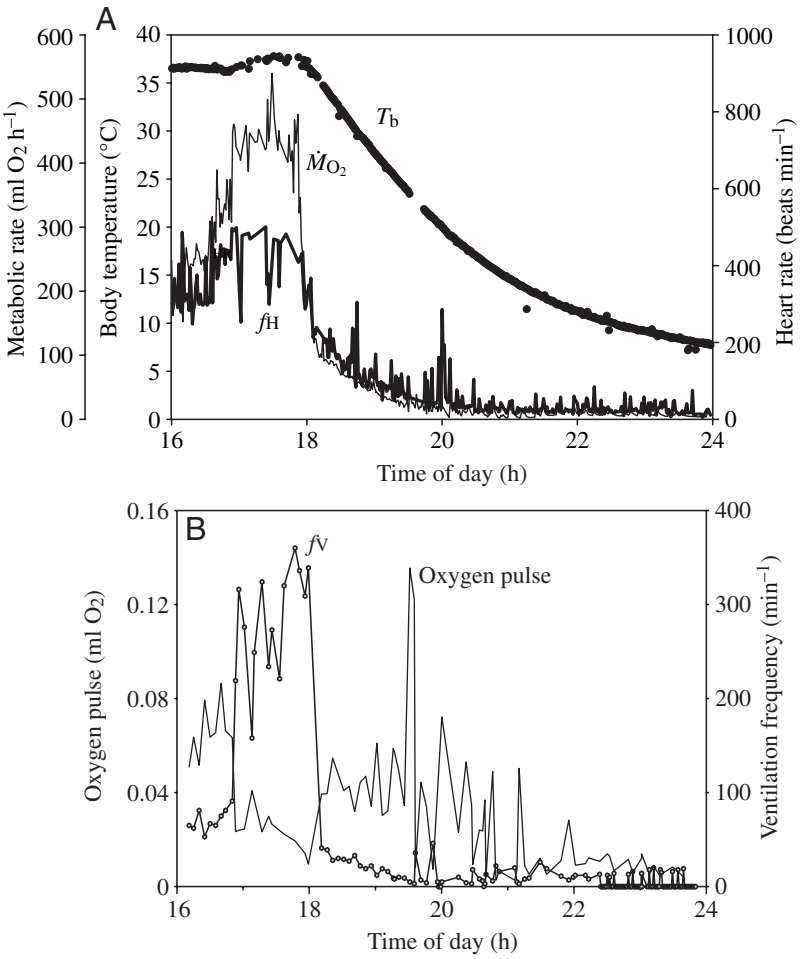

Fig. 5. (A) Sample recording at T_a=5°C of dormouse #L6S from 22.01.2001, BW=118 g. T_b (black filled circles), $\dot{M}_{O_2}$ (solid black line) and f_H (thick solid black line) were recorded simultaneously. The inset diagram further shows the oxygen pulse for ventilation, calculated from f_V and oxygen consumption. Note the change in scaling for f_V and $\dot{M}_{O_2}$ compared with Fig. 2. Scaling is adapted to show the consistence of congruent decline of $\dot{M}_{O_2}$ and f_H during entrance into torpor at different T_a. (B) The same entry into torpor as in Fig. 5A, but additionally the ventilation frequency (f_V, gray circles) and the oxygen pulse for ventilation are shown (compare Fig. 2B). Note the high ventilatory frequency and the simultaneous low oxygen pulse occuring prior to entrance into torpor.

Preparation for entrance into torpor

Immediately prior to metabolic depression dormice increased their metabolism, ventilation and heart rate (phase II). This period of enhancement lasted for about one hour, occurred regularly at each ambient temperature, and may thus serve as a necessary preparation for the following depression of metabolic rate. It was characterized by high ventilation frequency (up to 260 breaths min^{-1}), which increased from the resting level (phase I) of about 64 to 114 breaths min^{-1} (Fig. 3). The high frequency of ventilation in combination with low pressure changes during ventilation suggests that dormice showed a breathing pattern that closely resembled the breathing pattern of panting. Withers already observed in pocket mice (*Perognathus longimembris*) hyperventilatory phases that occured during entry and arousal when exposed to T_a between 5–10°C, and a correlation of decreasing respiratory parameters with the diminution of oxygen consumption (Withers, 1977). Metabolic and respiratory adjustments have also been observed for other hibernating mammals (Malan et al., 1973; Landau and Dawe, 1958; Kristoffersson and Soivio, 1964). Malan even noticed that the large increase of ventilation that seemed to characterize the beginning of an arousal in marmots was not accompanied by any significant increase of oxygen consumption that occurred later in the arousal process. The continuously recorded data set of ventilation frequency in dormice entering torpor indicates ventilation as an instantaneous and most sensitive parameter for changes of physiological states.

Metabolic and heart rate of dormice increased during the period of enhancement in phase II by about 80% above the resting values (Fig. 3) while body temperature hardly showed a reaction. To illustrate the change of metabolic rate, heart rate and body temperature mean values of phase I and phase II that preceded entries into torpor were plotted against different T_a values (Fig. 7). Metabolic and heart rate of a sample dormouse increased with decreasing T_a. An elevation from phase I to phase II could be measured throughout the whole ambient temperature range. The oxygen pulse for heart beat increased in parallel with metabolic rate to an elevated level during phase II (Table 1). Simultaneously, the oxygen pulse for ventilation in the pre-torpor phase, i.e. the amount of oxygen consumed per breath was reduced by 50%, underlining the suggestion of a panting like breathing pattern. This indicates an increased heart metabolism that is maintained elevated during the reduction phase and in deep torpor. These results confirm former studies on hibernating dormice or woodchucks showing a significantly higher blood flow to the heart during hibernation compared to other tissues or organs (Wells, 1971; Burlington et al., 1971). A more recent study performed on arousing Syrian hamsters (*Mesocricetus auratus*) underlines this assumption (Osborne and Hashimoto, 2003). The authors demonstrate that during arousal from hibernation large thermal gradients exist within the body of the hamster that probably result from coordinated, temporally specific restriction of blood to specific organs.

Peaks in metabolic rate prior entrance into torpor have already been observed in continuous recordings of pocket mice (*Perognathus longimembris*, Withers, 1977), Djungarian hamster (*Phodopus sungorus*, Heldmaier et al., 1999) and alpine marmots (*Marmota marmota*, Ortmann and Heldmaier, 2000). Here we showed that this peak occurred in dormice during more than 140 investigated spontaneous entries into torpor and was always associated with an increase in heart rate

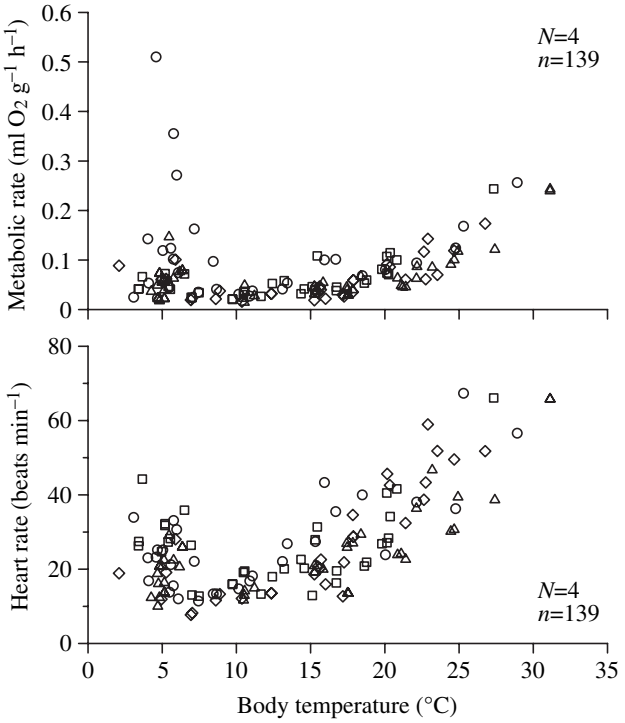

Fig. 6. Minimum values of deep torpor (phase IV) correlated with T_b (N=95). Top: minimum $\dot{M}_{O_2}$, bottom: minimum f_H. Individual dormice are marked with symbols: circles #R4S, squares #L6S, triangles #R13G, diamonds #R3S.

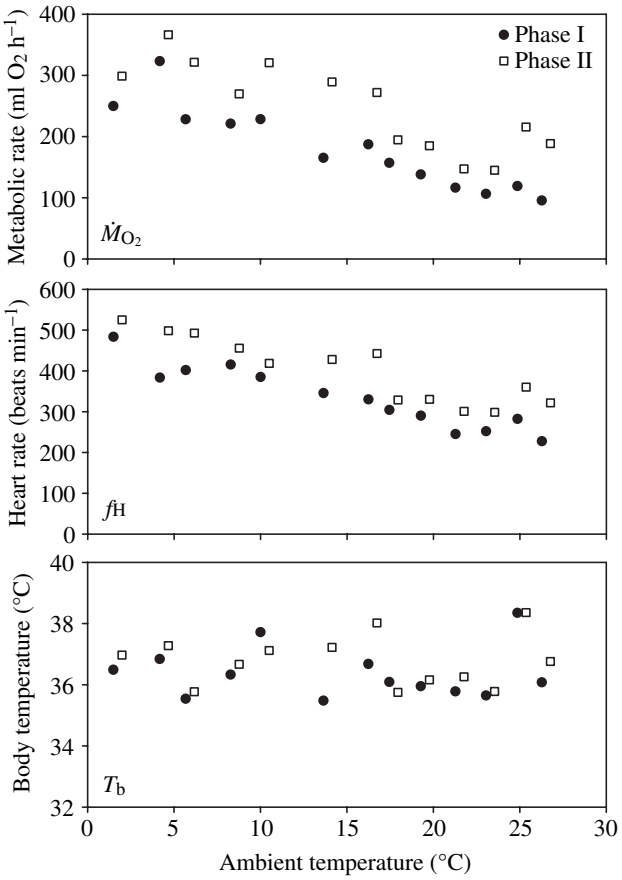

Fig. 7. Mean values of phase I and phase II preceeding entries into torpor of a sample dormouse #R3S at different ambient temperature. To elucidate the temporal delay between both phases the values of phase II are slightly displaced.

and ventilation frequency. The period of enhancement (phase II) seems to be the terminating process for eumetabolism and normothermia and simultaneously the preparatory step for an active, voluntary depression into torpor. The necessity of this preparation remains an open question, but it is possible that molecular or endocrinological mechanisms are switched on or off to facilitate the physiological depression.

Ventilatory, metabolic and heart rate depression: key parameters for entrance into torpor

During phase III, the actual entrance into torpor, metabolic rate, heart rate and ventilation are rapidly depressed. Almost instantaneously the dormice terminated the high rates observed during phase II, returned to resting levels, passed them and decreased their metabolic function in an exponential manner towards torpor levels. The present findings in dormice revealed that the decline of metabolism, heart rate and ventilation always occurred in parallel and was independent from ambient or body temperature. A close correlation between metabolic and heart rate has been confirmed in several studies (Morhardt and Morhardt, 1971; Butler et al., 1992; Bevan et al., 1994; Boyd et al., 1999). Milsom et al. (1999) postulated that the change in metabolic rate is mirrored by the change of heart rate and heart stroke volume.

Body temperature of dormice decreased during entrance into hibernation. Theoretically the decrease of body temperature could also be caused by an increase in the rate of heat loss, i.e. thermal conductance as it was suggested by Snyder and Nestler (1990). Previous studies on dormice revealed that the thermal conductance is not raised or reduced in daily torpor, hibernation or estivation (Wilz and Heldmaier, 2000). They calculated a mean minimum thermal conductance during entrance into torpor of 0.056 ml O_2 g^{-1} h^{-1} $°C^{-1}$, which is not different from the conductance of normothermic dormice of the present study (C=0.063 ml O_2 g^{-1} h^{-1} $°C^{-1}$). A similar constancy of thermal conductance was also measured during entrance into daily torpor in Djungarian hamsters (Heldmaier and Ruf, 1992). This indicates that an increase in heat loss is not involved in the transition into torpor. Instead thermal conductance is kept constant at a minimum level causing a slow decline of body temperature. The decrease of T_b lagged behind metabolic reduction and minimum body temperature was reached about 6 h after metabolic functions had reached their minimum. Ground squirrels (*Citellus tridecemlineatus*) similarly decline breathing rate, heart rate and body temperature while the fall of T_b lagged behind the drop of breathing and heart rates (Landau and Dawe, 1958). In woodchucks (*Marmota monax*) entering hibernation spontaneously Lyman (1982) observed a simultaneous decrease of heart rate with metabolic rate, and they also

Table 1. *Oxygen pulse for ventilation or heart beat*

	Metabolic rate (ml O_2 g^{-1} h^{-1})	Heart rate (beats min^{-1})	Oxygen pulse for ventilation (µl O_2 $breath^{-1}$)	Oxygen pulse for heart beat (µl O_2 heart $beat^{-1}$)
Phase I	1.42±0.05	314.8±69.0	54.5±14	9.3±2.0
Phase II	1.99±0.59	372.3±67.9	27.4±0.7	11.8±0.8
Phase III	0.265±0.087	169.1±35.53	26±4.9	6.05±1.62
Phase IV	0.017–0.25	8.2–66	0.562–0.9	4.9±1.5

Mean values of oxygen pulse for ventilation and oxygen pulse, or oxygen consumption, for heart beat (±S.D.). Phase I (N=5, n=31), phase II (N=3, n=11), phase III (N=3, n=10) and for heart and metabolic rate. Note that oxygen pulse for ventilation during steady state torpor (phase IV) is calculated from Wilz (1999). It is further important to mention the passive gas exchange during apnea while hibernating (Wilz et al., 2000).

reached hibernation values several hours before body temperature. In alpine marmots (*Marmota marmota*) minimum $\dot{M}_{O_2}$ is achieved after 10 h, but body temperature virtually decreased throughout the entire hibernation bout until the beginning of the next arousal (Ortmann and Heldmaier, 2000). A similar relation between metabolism and body temperature was also observed in Djungarian hamsters (*Phodopus sungorus*), indicating an active suppression of metabolic rate, and the decline of body temperatures could be interpreted as a consequence of the reduction of metabolic heat production (Heldmaier and Ruf, 1992; Heldmaier et al., 1999). Present data obtained from dormice entering torpor support these results – they even complete the known results of active metabolic suppression with a simultaneous depression of ventilation frequency and heart rate.

It has been suggested that the reduction of heart rate during entrance into torpor is under parasympathetic control (see Lyman, 1982), i.e. that heart rate is modulated by changing the balance between parasympathetic and sympathetic tone (Harris and Milsom, 1995). Atropine increases heart rate by slowing the effects of the parasympathetic nervous system while accelerating the effects of the sympathetic nervous system. Treatment of hamsters with atropine during entrance into hibernation elevates overall heart rate (Lyman and O'Brien, 1963; Zosky, 2002). Animals that have been atropinized before they begin to enter hibernation rarely succeed in entering hibernation. Lyman (1982) also observed in marmots that arrhythmias in heart rate caused by skipped or extra beats as occurred during reduction period disappeared with atropin treatment. Hence it was concluded that the increased parasympathetic activity modulates and decelerate heart rate (Lyman, 1982; Zimmer et al., 2000). An inhibition of vagal activity led to an increase of heart rate, even during apneic periods (Harris and Milsom, 1995; Zosky, 2002), which eliminated the breathing coupled tachycardia as described in dormice in Fig. 4 (Kristofferson and Soivio, 1964; Tähti and Soivio, 1975; Steffen and Riedesel, 1982; Grigg and Beard, 1996).

In *Spermophilus lateralis* it was shown that simultaneously to the decelerated heart rate the electrocardiogram was prolonged during deep hibernation (Steffen and Riedesel, 1982). At T_a=7°C they measured a duration from P to T wave of 0.062±0.09 s. A detailed analysis of ECG pattern revealed that dormice also develop arrhythmias during entrance into torpor and a prolonged ECG duration (Figs 8 and 9). At T_b=2.2°C the PT-duration lasted more than one second (Fig. 9A) while during normothermia it was maintained constant at 0.065±0.0067s over a wide range of T_a (Fig. 9B). The occurance of extra systoles and the prolongation of the ECG signal supports the assumption that the decrease in heart rate in dormice is also under control of parasympathetic tone. This suggests that parasympathetic activation and the initial changes in heart rate may be generally necessary for entrance into a torpid state (Milsom et al., 1999; Zimmer et al., 2000). Milsom and colleagues (1993) showed that stimulation of the vagus nerve reduced the mean heart rate by about 80% in normothermic as well as in hibernating squirrels (*Spermophilus lateralis*). Parasympathetic tone was also involved in homeostatic regulation during deep hibernation (Harris and Milsom, 1995), but it was emphasized that the reduction in body temperature associated with hibernation did not block vagal conduction.

Data of the present study support a paradigm that entrance into torpor is characterized by a controlled reduction of metabolic, cardiac and ventilatory activity. Several studies suggested that hypercapnia and hypoxia may induce or facilitate entry into torpor by suppressing metabolism (Studier and Baca, 1968; Williams and Rausch, 1973; Schäfer and Wünnenberg, 1976; Kuhnen et al., 1983). But there are also contrary observations described in the literature. Withers noticed that the pocket mouse exposed to T_as of 5–10°C spontaneously entered torpor even with *ad libitum* food. But no facilitation of entry was observed breathing 6% CO_2 (Withers, 1977). He assumed that hypercapnia and hypoxia are unlikely to be considered as important factors for the initiation of torpor, but this was not apparent for *P. longimembris*. The present study on dormice focussed on natural and undisturbed entries into torpor. Although we never exposed them to hypercapnic or hypoxic conditions they easily enter torpidity when being food reduced within a temperature range of 0–28°C T_a. However, we observed fluctuations in the RQ during entries into torpor within 0–7°C T_a (Elvert and

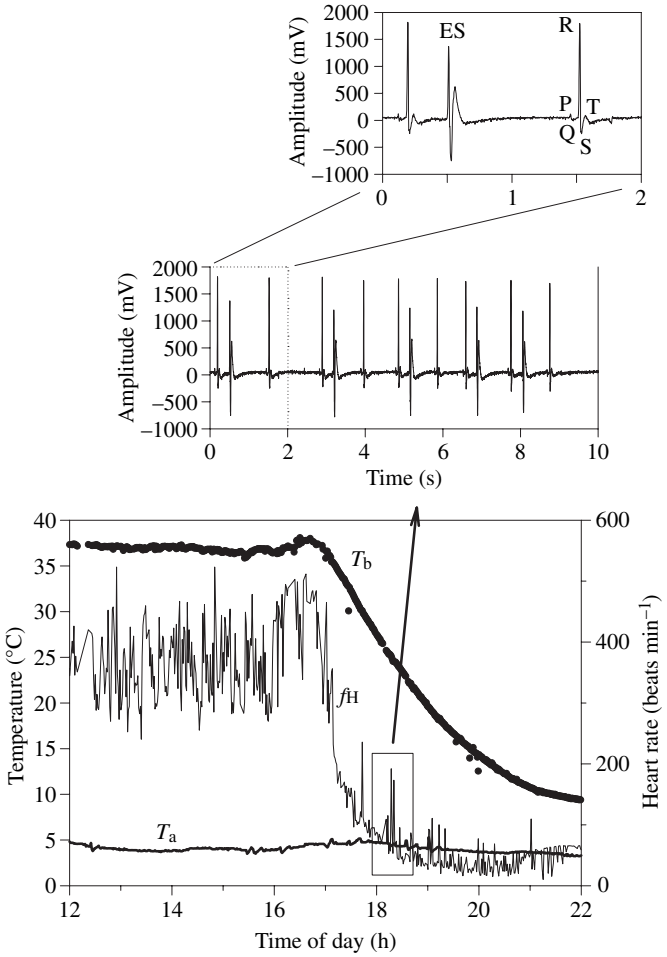

Fig. 8. The plot shows T_b (solid circles), f_H (thin solid line) and T_a (thick solid line). A sample peak in f_H during entrance into torpor is framed, indicating arrhythmia with extra systoles shown in the ECG plots above. Regular heart beats are interrupted by additional beats (ES). At the time of the snapshot the T_b was at 24.5°C.

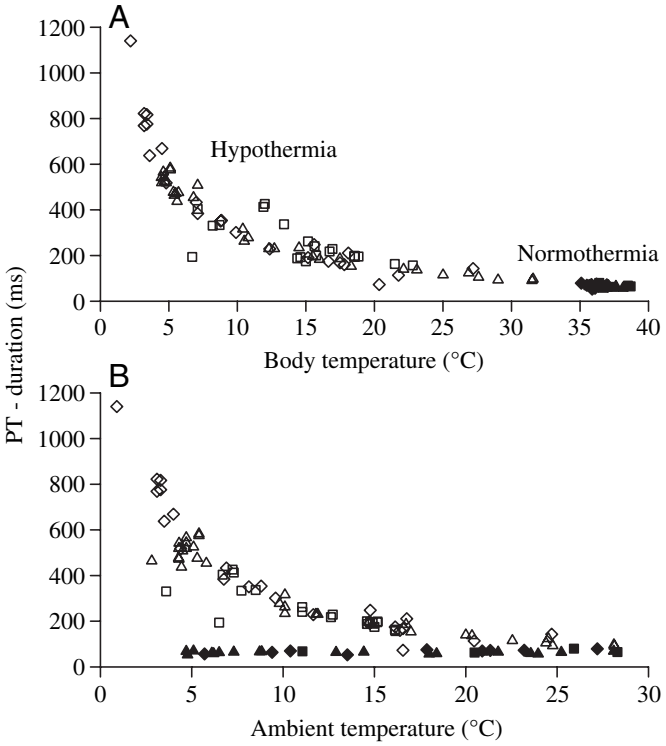

Fig. 9. The cardiac cycle length expressed as PT-duration as a function of T_b (top) and T_a (bottom) during normothermia (filled symbols, N=3, n=32) and hypothermia (open symbols, N=3, n=76). Individual dormice are marked with different symbols: squares #L6S, triangles #R13G, diamonds #R3S.

Heldmaier, 2000b). But these fluctuations were not caused by hypercapnia or hypoxia. The decrease of RQ closely paralleled the decrease of T_b in phase III. During normothermia and under food restriction the RQ was about 0.7 indicating a preferential combustion of lipids and was not related to hyperventilation or increased metabolic or heart rate during phase II. Bickler (1984) and Malan (1988) observed that the entrance into daily torpor or hibernation is accompanied by a falling respiratory exchange rate und suggested that this contributes to a CO_2 retention. Drops of RQ at the beginning of entry into torpor have been observed for several other species (Snapp and Heller, 1981; Bickler, 1984; Malan, 1986; Nestler, 1990). Hence, inhibitory effects of CO_2 retention and respiratory acidosis on thermoregulatory structures, glycolysis, neural activity and brown fat thermogenesis have been discussed (Malan et al., 1973). But there is still no evidence that indicates carbon dioxide retention generally acts as a stimulating factor for entrance into hibernation under normoxic conditions. There may be an accumulation of carbon dioxide in body fluids during entrance into torpor at very low ambient temperatures, as was described for dormice (Kreienbühl et al., 1976; Elvert and Heldmaier, 2000b). Blood analyses have shown that when the temperature of the blood decreased, its pH increased and P_{CO_2} decreased (Musacchia and Volkert, 1971; Kreienbühl et al., 1976; Rodeau and Malan, 1979; Malan, 1982). To maintain a constant P_{CO_2} and pH, additional storage of large quantities of CO_2 are required (Malan, 1982). Hence, it seems that CO_2 retention contributes to the control of a constant pH at low temperatures as it was discussed for dormice (Elvert and Heldmaier, 2000b). This further indicates a relative respiratory acidosis (Bharma and Milsom, 1993). It is thus unlikely for hypercapnia or hypoxyia to be the cause of metabolic depression but possibly facilitates a continuous decrease into deep hibernation.

The present data suggest that the transition into torpor is initiated by an endogenously programmed and interacting pattern of physiological inhibition, with actively downregulated ventilation, metabolic rate and heart rate to the low level in torpor. This study demonstrates that the entrance into torpor is a complex, but well coordinated interacting system, showing shifts in neurological control and changes in acid-base balance. But a detailed endocrine or neural signalling of overall depression as well as the function of the preparatory phase of hyperventilation and increased activity is not known. A clarification of mechanisms initiating the entrance into

torpor might be possible when focussing on the preparatory phase.

Abbreviations

C	conductance
f_H	heart rate
$\dot{M}_{O_2}$	metabolic rate
RMR	resting metabolic rate
RQ	respiratory quotient
T_a	ambient temperature
T_b	body temperature
f_V	ventilatory frequence
$\dot{V}_{O_2}$	rate of oxygen consumption

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