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Thermogenesis and Motor Recruitment of the Pectoral Muscle During Shivering in Arousing Bats *Murina Leucogaster*

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Temperate-resident bats exhibit a circadian cycle of torpor and arousal in summer. The physiological role and selective advantage of torpor as an energy saving mechanism have been received much attention by hibernation biologists. However, despite the significance of the recovering euthermic function, the arousal process and mechanism in these animals have been poorly addressed. In this study, we investigated thermogenic and motor activities of a local bat species *Murina leucogaster* during arousal by simultaneously examining oxygen consumption rate, body temperature (T_b) and pectoral electromyography (EMG). We found that T_b of the torpid bats (12-14℃) was augmented slowly by nonshivering mechanism during the initial awakening phase. The pectoral shivering, gauged by EMG activity, occurred between 17℃ and 38℃. Over this T_b range of shivering, heat was produced at a rate of 0.145 kcal kg^{-1} min^{-1} to raise 1℃ T_b per min. Shivering was most intensive at 30-35℃ where both EMG amplitude and spike frequency were the highest. Activation of the pectoral myofibers seemed to be controlled in a manner that motor units were recruited from smaller to larger sizes, with greater synchronization, as muscle shivering became intensive with increasing T_b.

Bats in the temperate region exhibit a daily cycle of activity in the summer (Altringham, 1996). They fly, feed, and maintain social activities at night, but usually retire into torpor in cool hibernaculum in daytime. Body temperature (T_b) fluctuates according to the daily activity cycle. For instance, Ognev's great tube-nosed bats, *Murina leucogaster ognevi*, show T_b of about 10-13℃ in torpor but 38-40℃ in flight (Choi et al., 1998a). Torpor, which represents hypometabolic and hypothermic status, is precisely regulated for energy-saving. In contrast, bats must make an enormous metabolic trial to raise T_b against the gradient of the hibernacular temperature in every arousal bout (Lyman, 1970; Altringham, 1996). The regulatory mechanism should be able to operate even at low T_b and accelerate heat production in a short period of time (Kulzer, 1967). Our previous study demonstrated that isometric tension of the *in vitro* biceps brachii muscle was maintained at tissue temperatures between 10℃ and 40℃ (Q_{10} = ~1.0), whereas the *in vivo* shivering response occurred at T_b above 17℃ during arousal (Choi et al., 1998a). This indicates that neural control for shivering initiates several degrees higher than the pre-arousal T_b, further suggesting that autonomic neural control for nonshivering is functioning prior to the onset of shivering.

In bats, nonshivering thermogenesis proceeds by oxidation of brown fat deposited in the interscapular region. Numerous mitochondria are found in the brown adipose tissue (BAT), that produce a large amount of heat for T_b augmentation (Lyman, 1970). The venus blood passing through BAT flows directly into the heart, and then raises temperatures of critical organs (e.g, brain, respiratory organ, muscles) (Schmidt-Nielsen, 1990). The oxidation process is known to be controlled by the neuro-endocrine coordination that is rather slow in effect.

To raise T_b rapidly against the large thermal gradient during arousal, a shivering mechanism by motor unit

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control would be more effective in bats. The pectoral muscle is known as the major site of shivering thermogenesis (Lyman, 1970). This muscle is uniformly composed of type IIc fibers and contains abundant mitochondria and lipid droplets around capillaries, an adaptive feature for high rate of prolonged contraction and energy yield (Ohtsu et al., 1978; Brigham et al., 1990). Furthermore, the muscle has a high capillary-to-fiber surface interface that would serve to enhance oxygen and nutrient transfer to muscle fibers (Mathieu-Costello et al., 1992). Thus far, structural properties and thermogenic potential of the pectoral muscle and BAT have been reported in these hibernators. However, contribution by shivering and nonshivering to the thermogenic process and motor recruitment patterns during pectoral shivering have been poorly understood. In this study, we simultaneously examined metabolic rate, change in T_b, and the pectoral electromyogram using a local bat species, *Murina leucogaster*. Our aims were (1) to find differential control of nonshivering and shivering mechanisms in the arousal phase, (2) to establish the relationship between rates of T_b increase and of heat production, and (3) to delineate recruitment pattern of motor units during the pectoral shivering.

Materials and Methods

Animals

We collected male bats *Murina leucogaster* from a natural cave located in Chiak Mt. (37° 20′ N, 128° 10′ E), Kangwon-Do, South Korea between June and September 1997 and 1998. Two bats were captured each time at dusk using bird nets when the animals were actively flying. In the laboratory, the bats were kept individually in dark, humid cages (length × width × height = 15×15×20 cm; ca. 15℃) for that night with water, pieces of apples and boiled albumin of eggs.

Rectal temperature measurements

To attain a general scope of thermogenic and shivering activities, we measured rectal temperature (T_r) of individual bats and recorded, on our visual bases, specific T_r's at which shivering and flight activities occurred. Each torpid animal was sat on a soft tissue pad in a cardboard box while a sensing tip of a 30-AWG duplex copper-constantan thermocouple was inserted 10 mm deep to the subject's cloaca. The thermocouple was connected to an Omega HH-73T digital thermocouple thermometer, and T_r was recorded at every 2 min interval until the T_r would no longer increase. The ambient temperature in the cardboard box ranged 20-22℃ throughout the measurements.

Preparation for thermogenic and electromyographic examination

On the day following captivity, one of the bats was weighed and anesthetized with thiopental sodium (0.001 mg g^{-1} intraperitoneally). Small cuts were made on the dorsal skin near the scapular and on the ventral skin of the left central chest for implantation of thermocouple and electrodes. A teflon-insulated duplex copper-constantan thermocouple (0.025 mm diameter, California Fine Wire, Grover City) was used to monitor T_b of the subject. The sensing tip was inserted about 5 mm deep in the central spot of the pectoral muscle via a 26-gauge hypodermic needle. To study EMG activity of the pectoral muscle during shivering, we used bipolar stainless steel electrodes and one ground electrode (0.051 mm diameter, California Fine Wire, Grover City) with uninsulated tips of about 0.5 mm. The bipolar electrodes were inserted right below the thermocouple in the pectoral muscle via the same hypodermic needle. The ground electrode was placed in the intraperitoneal cavity. Once implantation was completed, the bat was left in the cage (~25℃) for about 5 h of recovery under a lamp, and was allowed food and water *ad libitum*. The bat was then placed in a refrigerator (7℃) for a day to induce deep torpor.

Experiments

Every experiment began at 19:00, that roughly matched the daily activity cycle of these bats. We moved the torpid bat to a metabolic chamber (1 *l* in volume). The chamber temperature was maintained at 12±1℃ by a refrigerated circulator (Kookje Scien 33-WBF-15). The animal was allowed to perch in its normal (vertical) posture on a wire-mesh wall installed inside the chamber. The thermocouple and the electrodes inserted in the bat were taken out through the top of the chamber. The thermocouple was connected to an Omega HH-73T digital thermometer. The electrodes were connected to a Grass P511 preamplifier by which EMG signals were amplified 10,000x. Half-amplitude low filter and half-amplitude high filter of the preamplifier were set at 100 Hz and 3 kHz, respectively, and 60 Hz notch filter was turned on.

Oxygen consumption rate (V_{O_2}) was measured with an Oxymax system (Columbus Instrument, Ohio) that included an automated, open-circuit equal flow system set at 500 ml min^{-1}. Incurrent air flow was controlled by the air supply regulator of the system (overall capacity of 0-2450 ml min^{-1}). The excurrent subsample of air (100-150 ml min^{-1}) was diverted through a couple of drier columns to the O_2 sensor. The gas analyzer was calibrated on each day of experiment.

After an equilibration for 30 min in the chamber, we awoke the subject by gently touching the back of the animal 5 times (for 5-10 sec) with a 1-mm thick steel rod. The rod was introduced through a hole of the chamber top. Right after the last touch, we recorded T_b, metabolic rate and EMG simultaneously. V_{O_2} signals from the oxygen sensors were transmitted every 2 min through an Oxymax A/D converter to an IBM Pentium

compatible PC. The thermocouple reading for T_b was also recorded at each time when Vo_2 was recorded. EMG signals from the preamplifier were digitized via a Biopac MP100 A/D converter and monitored by another Pentium PC. One-second length EMG signals were collected and stored as soon as the bat's T_b reached 20℃, 25℃, 30℃, 35℃, and those where the signals were first and last detected during arousal (e.g., around 17℃ and 37℃).

Data analysis

To evaluate thermogenic effort of the subjects during arousal, we related the rate of heat production (RHP, kcal kg^{-1} min^{-1}) with that of T_b increase (RT_b, ℃ min^{-1}). RHP was calculated from an equation, $RHP=4.6 \times Vo_2$ where Vo_2 had the unit of O_2 l kg^{-1} min^{-1} (Schmidt-Nielsen, 1990). From the raw EMG signals at each T_b, peak amplitude and median frequency were analyzed using a software ACK100W (Biopac systems, Inc. Goleta, CA). Peak amplitude provides intuitive information on numbers and sizes as well as the extent of synchronization of active motor units. Median frequency of the signals allows evaluatation of recruited or synchronized level of the active motor units. Data are presented as mean±SD, unless otherwise noted. Significance of the RHP-RT_b relationship was tested by the *t*-test on Pearson's *r*. Statistical significance of EMG activities over the six different T_b's was examined by one-way Scheffe's multiple range tests. All statistical procedures were performed with SPSS+/PC (SPSS Inc.).

Results and Discussion

Body mass (M_b) and change in T_b and in Vo_2 were summarized in Table 1. Changes in T_r of the subjects over the time course of arousal were illustrated in Fig. 1. The bats increased T_r from 15.3±0.1℃ to 37.6±0.8℃ in 35-38 min. Shivering response of the bats began at around 17-18℃ and flight activity at 28-30℃. These T_r's for the corresponding activities were comparable to those observed in our previous study (Choi et al., 1998a).

It turned out that simultaneous recordings of T_b, Vo_2, and EMG signals were prone to frequent experimental failures due to breakage or pull-off of EMG electrodes and thermocouples, or death of subjects in the middle of trials. Typical recordings of T_b, metabolic rate, and the pectoral EMG activity of a bat were presented in Fig. 2. Note that thermogenesis started in the non-shivering way after stimulation, and that slow increase

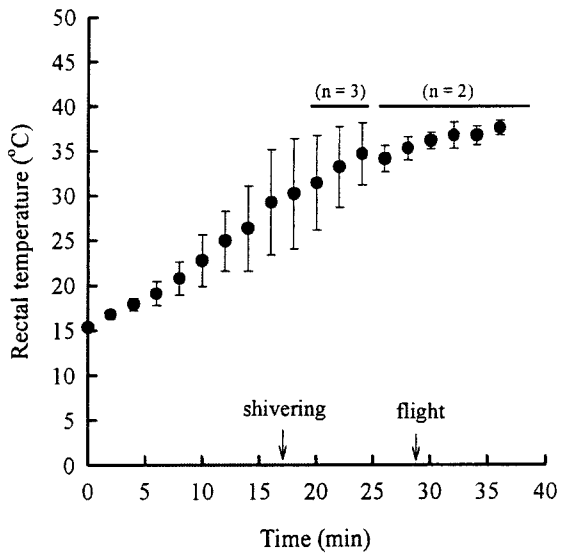

Fig. 1. Change in rectal temperature over the time course of arousal in *Murina leucogaster*. Due to flight escape behavior of the bats at T_b's > 28℃, the initial sample size of 7 was reduced to 2-3 in cases of the late time course.

in Vo_2 and T_b was observed during the initial phase of awakening. The pectoral shivering (gauged by EMG activity) began at T_b average of 17.4±0.7℃, from which it was the major thermogenic source for rapid T_b increase (Table 1 and Fig. 2). The greatest rate of T_b increment was seen between 25℃ and 35℃ where the metabolic rate reached near maximum (13.37 l kg^{-1} h^{-1}). The Body temperature continued to rise more than 4-5℃ even after the metabolic rate was being lowered (Fig. 2).

After the complete arousal, T_b reached to 38.2±0.6℃. This T_b was slightly lower than the highest T_b of bats captured in flight (40℃, Choi et al., 1998a), suggesting that the flight activity exacted the bats' pectoral muscle to produce additional heat. The highest Vo_2 (13.37 l kg^{-1} h^{-1}) in maximal shivering was comparable to the value that was recorded at 5℃ T_a (near the coldest hibernacular temperature in winter), but was 1.45-fold greater than that examined at 15℃ T_a (close to the hibernacular temperature in summer) in these bats (Choi et al., 1998b). This result implies that the subjects utilized their maximal thermogenic capacity to augment T_b at the highest rate during arousal (1.21℃ min^{-1}; Table 1). The average rate of T_b change was 0.77 ℃ per min, which led the arousing subjects to recover T_b of their active state (38℃) in approximately 30 min.

Fig. 3. illustrates a relationship between rates of heat production (RHP) and of T_b increase (RT_b) from 17℃

Table 1. Thermogenic and metabolic properties of *Murina leucogaster* males during arousal

M_b (g)	Peak T_b (℃)	Peak Vo_2 (l kg^{-1} h^{-1})	mean change in T_b per min	maximum change in T_b per min	maximum change in Vo_2 per min
7.4±0.8* (9)	38.2±0.6 (6)	13.37±1.50 (4)	0.77±0.36 (5)	1.21±0.15 (5)	1.39±0.15 (4)

* Values are mean±SD. Sample size for each variable is in parenthesis. Discrepancy in sample sizes among variables resulted from experimental failure in several cases during EMG recordings. M_b; body mass, T_b; body temperature, Vo_2; oxygen consumption rate.

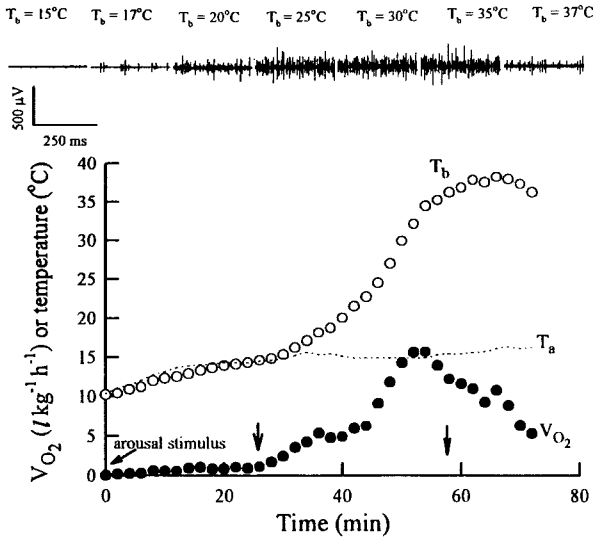

Fig. 2. A typical illustration showing changes in oxygen consumption rate (V_{O_2}), body temperature (T_b), and the pectoral EMG activity (at seven different T_b) in an arousing bat, *M. leucogaster*. Two arrows on the time axis indicate points of initiation and termination of the muscle shivering. Note parallel changes in V_{O_2}, T_b and the EMG activities. T_a is the chamber temperature initially set at 13℃ and increased slightly as the subject's T_b increased.

to 38℃. The two variables showed a positive linear relationship with a regression equation, RHP = -0.018 + 0.163 RT_b ($r = 0.895$, $P < 0.05$). This relationship indicates that over the period of pectoral shivering the subjects produced heat at a rate of 0.145 kcal kg^{-1} min^{-1} to raise 1℃ T_b per min. An overall amount of heat required for the bats to raise T_b against the thermal gradient of 25℃ for 30 min was estimated to be 108.75 kcal kg^{-1}. This amount of heat (generated for 30 min) is about 12% that of daily energy requirement in these small-sized bats, considering that daily energy expenditure of the 8 g little brown bats *Myotis lucifugus* is estimated to be 886 kcal kg^{-1} d^{-1} (Altringham, 1996).

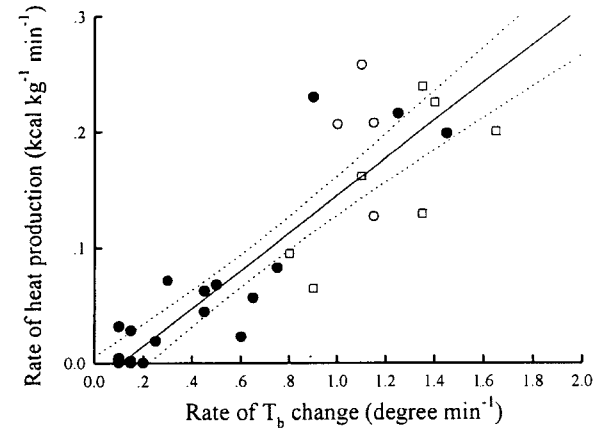

Fig. 3. Relationship between rate of heat production (RHP, kcal kg^{-1} min^{-1}) and rate of T_b increase (RT_b, degree min^{-1}) examined at T_b's between 17℃ and 38℃ in arousing *Murina leucogaster*. The two variables showed a positive linear relationship with a regression equation, RHP = -0.018 + 0.163 RT_b ($r = 0.895$, $P < 0.05$).

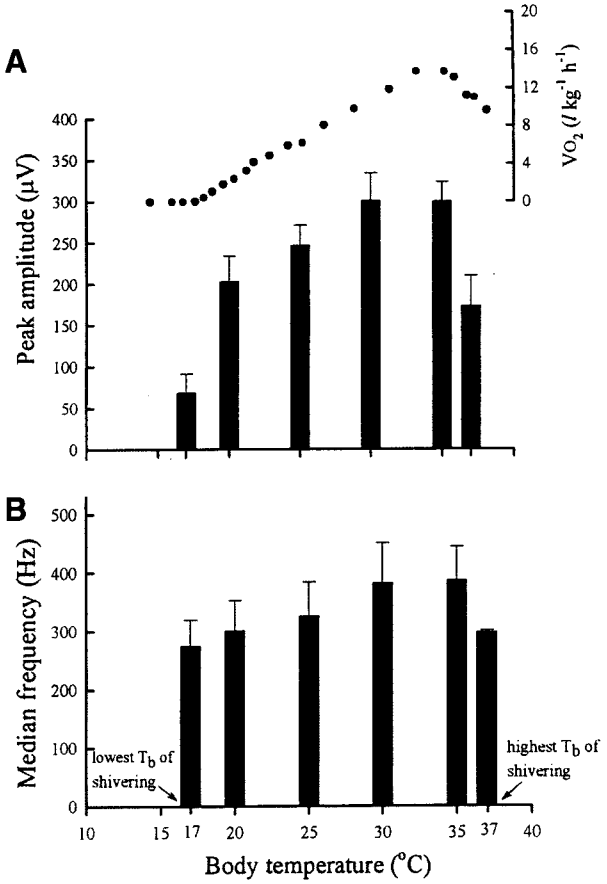

Fig. 4. Change in the EMG activity at six different T_b's during arousal in *Murina leucogaster*. Both peak amplitude (A) and median frequency (B) increased with T_b and reached highest at 30-35℃. The average metabolic rate (n = 4) is shown in the uppermost panel for reference. Shivering was not detected below 17℃ and above 38℃.

In our EMG study (Fig. 4), both peak amplitude and median frequency changed significantly over the T_b range between 17℃ and 38℃ (one-way Scheffe's multiple range, $P < 0.05$). The EMG amplitude increased rapidly between 17℃ and 20℃ (Fig. 4A), while the median frequency increased relatively slowly between the two T_b's (Fig. 4B). The EMG activity increased further with T_b, and reached the highest at 30-35℃. Motor units are known to be recruited in an order from smaller to larger sizes and from oxidative to glycolytic myofibers while the motor neurons increase their mechanical output (Rome et al., 1992; Herzog, et al., 1994). The increase in EMG amplitude and frequency with increasing T_b in our bats would demonstrate that motor units in the pectoral shivering were recruited from smaller to larger sizes with greater synchronization in activation. It is less likely that a shift in myofiber types from oxidative to glycolytic ones occurred during the shivering, because the pectoral muscle of bats is known to be composed of a similar-sized, uniform type (IIc) of myofibers within a season (Brigham et al., 1994; Kim et al., 2000).

In conclusion, thermogenic activity of the arousing bats occurred in a differential manner in that a non-

shivering control preceded a powerful shivering during the initial awakening phase. The pectoral shivering occurred most vigorously between 30℃ and 35℃ T_b, and generated heat of 0.145 kcal kg^{-1} per degree T_b per min. As the shivering thermogenesis increased, there seemed to be a shift in recruitment of motor units from small to large ones with greater synchronization in activation.

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