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ORIGINAL ARTICLE

Comparison of Nonshivering Thermogenesis Induced by Norepinephrine Stress in Tree Shrews

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Nonshivering thermogenesis (NST)is an important thermogenic mechanism for small mammals. Quantitative measurement of NST is usually stimulated by injection of norepinephrine. The injection dosage of norepinephrine (NE)is critical for eliciting the maximum NST. Three empirical equations of NE dosages were often referenced in previous studies: (1)NE (mg / kg)= $2.53W^{-0.4}$; (2)NE(mg/kg) = $3.3W^{-0.458}$ and (3)NE(mg/kg)= $6.6W^{-0.458}$. In the present study, we used tree shrews (*Tupaia belangeri*) as experiment subjects to test the effects of the three dosages of NE on NST. Results showed that all the three dosages of NE could induce the maximum NST for *T. belangeri*. No significant differences were found in NST among groups and the NST was respectively 2.63±0.12 (formula 1), 2.66±0. 11 (formula 2) and 2.78±0.15 (formula 3). However, when injected with NE dosage from formula 3, the increase of body temperatures was significantly higher than the other two NE dosages (increased 1.5±0. 1°C (formula 3), 0.8±0. 2°C (formula 2), and 0.6±0. 1°C (formula 1), respectively). In order to prevent the death because of hyperthermia, formula 1 or 2 is recommended to be used.

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Non-shivering thermogenesis occurs in brown adipose tissue (brown fat) that is present in all mammals, brown adipose tissue has a unique protein (uncoupling protein-1) that allows the uncoupling of electrons moving down their mitochondrial gradient from the synthesis of ATP, thus allowing the energy to be dissipated as heat (Jansky, 1973). Non-shivering thermogenesis is regulated mainly by thyroid hormone and the sympathetic nervous system. Some hormones, such as norepinephrine (NE), may stimulate thermogenesis by activating the sympathetic nervous system, Because NE induced NST by heat is equivalent to that induced by cold, therefore, the maximum NST is generally defined as the total metabolic responses to NE, which can injecte the NE dose related to the body mass, determination of animal in the following thermal neutral zone in a quiet, sober condition to determine the maximum oxygen consumption (Heldmaier *et al*., 1982).

Scholars focus on NST induced by different doses of NE on small mammals for many years, but only selected by the optimal NE injection dose obtain accurate and reliable results for NST. To determine appropriate doses of NE injection, which need a set of the different dose gradient, making dose response curve, and also need large amount of samples. Therefore, some scholars try to establish relations of NE injection dose related to body mass in small mammals. At present, there are 3 dose calculation formula in the literature: (1)NE(mg/kg)= $2.53W^{-0.4}$ (Wunder and Gettinger, 1996); (2)NE(mg/kg) = $3.3W^{-0.458}$ (Merritt, 1986) and (3)NE(mg/kg)= $6.6W^{-0.458}$ (Heldmaier, 1971). These 3 kinds of doses are applied, and they can induce the maximum NST capacity, but there is not a systematic evaluation of the 3 NE dose.

Tupaia belangeri (Mammalia: Scandentia: Tupaiidae) live at the highest latitude, with the Yunnan-Kweichow Plateau being its northern limit (Wang *et al*., 1991). Previous studies demonstrate that environmental factors, such as short photoperiods and cold, are effective cues that influence body mass and thermogenesis in *T. belangeri*, separately (Zhang *et al*., 2011; 2012a; 2012b; 2012c). *T. belangeri* shown a seasonal increased in body mass and thermogenic capacity to adapt to the increase of energy requirements for thermoregulation (Zhu *et al*., 2012). In the present study, we determined the optimal NE dose induced by NST by comparing the 3 dose calculation formula.

MATERIALS AND METHODS

Samples

T. belangeri were captured (25°25´-26°22´ N, 102°13´-102°57´ E, 1679 m in altitude) around boscage at Luquan County in 2012. The average yearly temperature was 15.6 °C, mean monthly temperature ranges from 7.8 °C in winter to 19.6 °C in summer. After being captured, *T. belangeri* were brought and bred at the School of Life Sciences, Yunnan Normal University, Kunming (1910 m in altitude). Each weight-matched tree shrew was housed individually in a wire cage (40 cm×40 cm×40 cm) with no bedding; all animals (60 males) were healthy adults. The photoperiod, ambient temperature and humidity were maintained at 12 L: 12D (light on at 0800 h), 25 (±1)°C , and 85%–92% relative humidity, respectively. Animals were kept for at least two weeks, and 10 adults with similar body mass were measured. 3 kinds of NE dose according to experiment the sequence from low to high: (1)NE (mg / kg) = $2.53W^{-0.4}$; (2)NE(mg/kg) = $3.3W^{-0.458}$ and (3)NE(mg/kg)= $6.6W^{-0.458}$. Injection interval different dose is 3 day in order to avoid the effect of NE on metabolism (Wang and Wang, 2006). Pregnant, lactating or young individuals were excluded.

Measurement of metabolic rates

Metabolic rates were measured by using AD ML870 open respirometer (AD Instruments, Australia) at 30°C within the TNZ (thermal neutral zone), gas analysis were using ML206 gas analysis instrument, the temperature was controlled by SPX-300 artificial climatic engine (±0.5°C), the metabolic chamber volume is 500 ml, flow is 200 ml/min. The tree shrew were stabilized in the metabolic chamber for at least 60 min prior to the RMR measurement, oxygen consumption was recorded for more than 60 min at 5 min intervals. Two stable consecutive lowest readings were taken to calculate RMR (Li and Wang, 2005). Calculate method of metabolic rate is detailed by Hills (1972) (Hill, 1972). All metabolic measurements were performed more than 400 hours. Before and after the experiment, animal's body mass were measured, and body temperature were measured by digital thermometer (Sinan instrument of Beijing Normal University, SN2202), 1.5 cm was the probe inserted to the rectum, and read the body temperature after 45 s.

Nonshivering thermogenesis (NST) was induced by subcutaneous injection of norepinephrine (NE) (Shanghai Harvest Pharmaceutical Co. Ltd) and measured at 25°C. Two consecutive highest recordings of oxygen consumption more than 60 min at each measurement were taken to calculate the NST (Zhu *et al*., 2010).

Statistical analysis

Data were analyzed using SPSS 15.0 software package. Prior to all statistical analyses, data were examined for assumptions of normality and homogeneity of variance, using Kolmogorov-Smirnov and Levene tests, respectively. Throughout the acclimation, NST were analyzed by Repeated measures ANCOVA with body mass as a covariate. Results were presented as mean ± SEM, and $P < 0.05$ was considered to be statistically significant.

RESULTS

Since no gender effects were found in any of the measured parameters, data from females and males were combined thereafter.

No differences of RMR were found between 3 kinds of NE does in *T. belangeri* ($F=0.693$, $P>0.05$, Table 1), there were also showed that No differences of RMR were found between 3 kinds of NE does ($F=1.322$, $P>0.05$, fig 1). For the body mass, there were no significant differences before and after the experiment (Table 1). Injected with NE dosage from formula 3, the increase of body temperatures was significantly higher than the other two NE dosages (increased 1.5±0. 1°C (formula 3), 0.8±0. 2°C (formula 2), and 0.6±0. 1°C (formula 1), respectively (fig 2).

Table 1. Body mass and resting metabolic rate (RMR) elicited by 3 different norepinephrine dosages in *Tupaia belangeri*

NE does	Initial body mass (g)	Final body mass (g)	RMR (ml O_2/g.h)
NE(mg / kg) = $2.53W^{-0.4}$	102.32±3.21	100.63±3.22	1.23±0.21
NE(mg/kg) = $3.3W^{-0.458}$	103.32±2.36	102.01±2.31	1.25±0.16
NE(mg/kg)= $6.6W^{-0.458}$	101.32±2.32	99.25±2.33	1.28±0.25

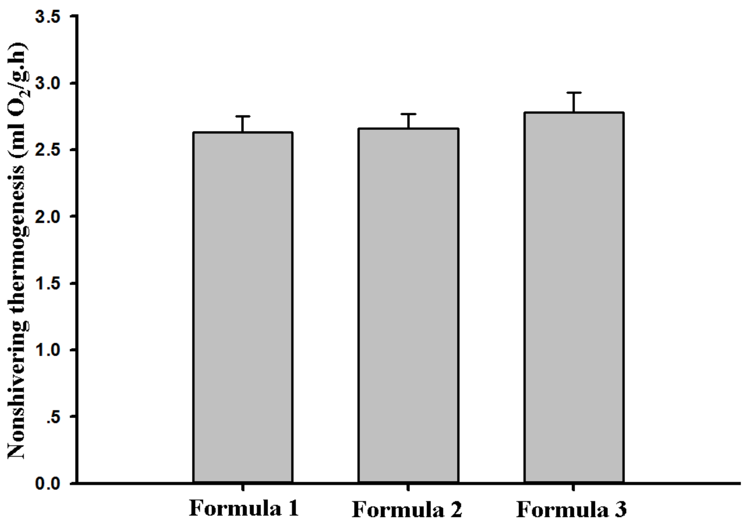

Figure 1. NST elicited by 3 different norepinephrine dosages in *Tupaia belangeri*

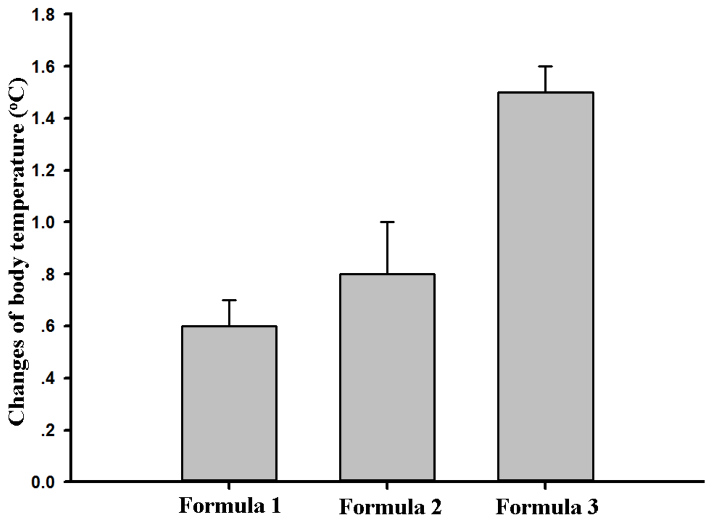

Figure 2. Changes of body temperature elicited by 3 different norepinephrine dosages in *Tupaia belangeri*

DISCUSSION

NST is an important way of heat production in small mammals (Heldmaier *et al*., 1990). Non-shivering thermogenesis is regulated mainly by the sympathetic nervous system (Klingenberg and Echtay, 2001). Due to NST associated with NE does, the optimal NE injection dose obtain accurate NST (Depocas, 1960). When the injection of NE dose were too low, NE heat production contribution will not be fully displayed, at high doses induced thermogenesis remains unchanged or even drop, while the animal always lose the ability to regulate the body temperature because of hyperthermia, eventually led to the death (Wunder and Gettinger, 1996). Heldmaier (1971) proposed formula (3) is widely used in heat production researches (Li *et al*., 2001). But because of the experimental animal was under 5℃ acclimation treatment, and the animal were anesthesia in the determination of NST, so it was generally believed that NE injection will cause the animal activity, and animal activity will affect the metabolism of determination, and later found anesthesia will produce some effects on metabolism, and after the injection of NE, animal often does not move. And many experiments were not at 5℃ domestication, therefore, application of this dose formula is limited. Wunder and Gettinger studied wild small mammals which body weight were 15-900g , the experimental domestication was 23℃, animal without anesthesia, the results showed that NE dose of acclimation temperature does not affect the induction of NST, but anesthesia will affect the NE sensitivity, and proposed formula (1) according to the dose response curve. In the present study, NST induced by 3 different norepinephrine dosages showed no significant differences in *T. belangeri*, which indicated that 3 kinds of injection dose can induce the maximum NST, but the changes of body temperature showed significant differences. Injected with higher NE dosage (formula 3), the increase of body temperatures was significantly higher than the other two NE dosages.

In conclusion, the present results suggested 3 doses can be used, we recommended dose (1) and (or) (2).

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