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ORIGINAL ARTICLE

Effect of Ambient Temperature on Body Temperature and Rest Metabolic Rate in *Apodemus chevrieri* During Postnatal Development

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In order to investigate the ability of constant temperature and thermoregulation in *Apodemus chevrieri*, body temperature and rest metabolic rate (RMR) were measured during postnatal development (1～42 day) when the *A. chevrieri* exposed different ambient temperature. The result showed that: body temperature and RMR of pups in *A. chevrieri* increased according to the increase of ambient temperature during 1 day to 7 day, showed character of poikilotherms; body temperature of pups were lower in low temperature(5°C and 10°C), relatively and RMR significant increased when day age is 14 day, it indicated that the pups showed a certain degree of thermoregulation in this phase. Its thermoregulation ability developed quickly during 7 day to 14 day. RMR of pups was extreme significantly higher in low temperature than that in other temperature when day age was 21 day, it showed that the pups had some thermoregulation to low temperature stimulation. The RMR of pups was showed increasing trend in high temperature(35°C) when 28 day; when day age was 35 day and 42 day, the thermal neutral zone were 22.5 to 30°C and approaching its adult level. All of these results indicated that pups of *A. chevrieri* in the different growing period had different thermogenesis and energy allocation to maintain stable to body temperature, thermogenesis was weaker in the early phase of postnatal development, most of energy is used to its growth. After pups were weaned, the ability of constant temperature and thermoregulation developed quickly to adjust variations of environment during postnatal development.

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Temperature is an important factor to influence growth and development in small mammals (Leon 1986), mother provides a constant environment during embryo period in endotherm, pups after birth will directly face the surrounding environment, acquisition and distribution of energy, heat capacity, ability to regulate body temperature and heat dissipation are threats to the survival of pups, therefore, rapid growth and improve the body function are very important during postnatal development (Krol and Speakman 2006). There had many researches about postnatal development, in accordance with the development of mammals are born with constant temperature mechanism, the pups were divided into 3 categories: precocial, altricial and immature (Brück and Hinckel 1996; Cannon and Nedergaard 2004). Unlike precocial pups, body surface of altricial pups was exposed, growth was not complete, many functions of the body is not coordinate and perfect, ability of physiological heat production was weak, which can not prevent heat loss effectively, had weak ability of the thermoregulation, body temperature began to decline when the ambient temperature is slightly lower than the thermal neutral zone, pups has a large dependence on parents after birth (Hull 1973). Unde the care of parents, pups will able to maintain a relative higher body temperature (Hill 1992), a higher temperature is beneficial to rapid growth (McManus 1971).

Daily temperature difference was larger in Hengduan mountains region, pups after birth are likely to experience of the high temperature stress, but also to experience of the low temperature stress in a very short period, although the pups had care and protective of parent, but in order to adapt to the environment, its body temperature and heat production adjustment also needs to have the rapid development. In the present study, body temperature and RMR were measured in pups of *A. chevrieri* under different temperature conditions, in order to understand the development process and its ability to heat the thermostat capability, further reveal adaptive features of Hengduan mountain region during postnatal development.

MATERIALS AND METHODS

Samples

Seven pregnant females were captured (26.22°N, 99.48°E, and 2, 550-2, 615m in altitude) around Jianchuan County in July 2010, then brought and bred at the School of Life Sciences, Yunnan Normal University, Kunming (1, 910 m in altitude). Each mouse was housed individually in plastic boxes (260mm× 160mm×150mm) with sawdust bedding with no nest under a constant light cycle (12:12 h light-dark cycle, lights 0900-2100) and temperature (25±1°C), and maintained on a commercial standard rat pellet (produced by Kunming Medical College). Food and water were available *ad libitum*. Relative ambient humidity ranged from 60% to 75%. There were 33 pups were used in the experiment, all animals were healthy individual.

Measurement of resting metabolic rates

Body temperature and RMR were measured every week from 1 day to 42 day in 5, 10, 15, 20, 25, 30 and 35°C (except for 35°C in 1 and 7 day). Metabolic rates were measured by using AD ML870 open respirometer (AD Instruments, Australia) at 25°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 500ml, flow is 200 ml/min. Animals were stabilized in the metabolic chamber for at least 60 min prior to the BMR measurement, oxygen consumption was recorded for more than 60·min at 5·min intervals. Two stable consecutive lowest readings were taken to calculate RMR. Calculate method of metabolic rate is detailed by Hills (Hill 1972). Body temperature were measured by digital thermometer (Sinan instrument of Beijing Normal University, SN2202), 1.5cm was the probe inserted to the rectum, and read the body temperature after 45s.

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, including analysis of covariance(ANCOVA) and repeat metrical regression and Two way-ANOVA. Results were presented as mean ± SEM, and $P < 0.05$ was considered to be statistically significant.

RESULTS

Body temperature

Time and ambient temperature had significant effect of body temperature on pups of *A. chevrieri* (time: F=2.171, P<0.01, ambient temperature: F=242.3, P<0.01 interaction between time and ambient temperature: F=41.48, P< 0.01, Table 1).

Body temperature in *A. chevrieri* changes gradually With the increase of age and ambient temperature. Pups of *A. chevrieri* elevated body temperature with ambient temperature rises on 1 day and 7 day, and close to the ambient temperature, showed similar characteristics of allotherm. At the age of 1 days, linear regression equation between body temperature (Tb) with ambient temperature (Ta) of pups in *A. chevrieri* is :$T_b = 0.64T_a + 9.37$ ($R^2 = 0.97$, $P < 0.01$); at the age of 7 days, linear regression equation between body temperature (Tb) with ambient temperature (Ta) of pups in *A. chevrieri* is :$T_b = 0.66T_a + 9.65$ ($R^2 = 0.93$，$P < 0.01$); at the age of 14 day, pups showed weak temperature regulating ability, and body temperature maintain relatively stable after 15°C; at the age of 21 day, minimum body temperature ,was: 30 ± 0.32°C in 20°C; at the age of 28 days, linear regression equation between body temperature (Tb) with ambient temperature (Ta) of pups in *A. chevrieri* is :$T_b = 0.11T_a + 33.67$ ($R^2 = 0.84$, $P < 0.01$); at the age of 35 days, linear regression equation between body temperature (Tb) with ambient temperature (Ta) of pups in *A. chevrieri* is :$T_b = 0.11T_a + 33.82$ ($R^2 = 0.80$, $P < 0.01$); at the age of 42 days, pups of body temperature are relatively stable after 15°C , showing stable body temperature.

With the increase of age, body temperature of pups in *A. chevrieri* gradually stabilized at the same temperature(Table 1). Body temperature in pups at before 28 days of age increased the with the increase of time, after age of 28 days, the body temperature difference is not significant in 5°C; body temperature are relatively stable after 28 days in 10°C, the difference was not significant (P>0.05); body temperature are relatively stable after 14 days in 15°C, the difference was not significant (P>0.05); similar changes found in 20°C and 25°C; the difference was not significant (P>0.05); body temperature are relatively stable after 14 days in 30°C; body temperature of pups at the age of 14 days and 21 days of age showed no significant difference (P >0.05), it had no significant difference after 28 days in 35°C(P >0.05).

RMR

Time and ambient temperature had significant effect on RMR in pups of *A. chevrieri* (time: F=207.6, P<0.01, ambient temperature: F=30.77, P<0.01 interaction between time and ambient temperature: F=17.97, P< 0.01, Table 2).

At the age of 1 days, linear regression equation between RMR with ambient temperature (Ta) of pups in *A. chevrieri* is: $RMR = 0.12T_a - 0.15$ ($R^2 = 0.80$, $P < 0.01$); at the 7 days of age, linear regression equation between RMR with ambient temperature (Ta) of pups in *A. chevrieri* is: $RMR = 0.12T_a+0.11$($R^2 = 0.80$, $P<0.01$); at the 14 days of age, pups in *A. chevrieri* increased heat production at low temperature, the maximum RMR appeared at 15 and 20°C, and RMR in pups showed no significant differences after 20°C; when the pups were 21 day old, the maximum RMR appeared at 5 and 10°C ($P < 0.01$), RMR had no significant difference after 15°C; at 28 days of age, along with the the increase of the ambient temperature, RMR decreased gradually, the lowest RMR appeared in 25°C and 30°C; at the age of 35 days, RMR were lower between 20°C and 30°C without significant difference ($P>0.05$); at 42 days of age, RMR has minimum value at 25°C, when environment temperature below 25°C, RMR decreased with the increase of ambient temperature.

Table 1. The body temperature of *A. chevrieri* pups that after exposure to different ambient temperature for 1h during postnatal development

Ambient temperature(°C)	Body temperature(℃)						
	1 d	7 d	14 d	21 d	28 d	35 d	42 d
5	12.08±0.21^{f}	13.86±0.44^{f}	30.44±0.42^{b}	36.16±0.47^{a}	34.32±0.27^{e}	34.58±0.35^{d}	35.18±0.46^{c}
10	16.78±0.40^{e}	16.44±0.17^{e}	31.06±0.43^{b}	35.40±0.15^{a}	34.98±0.18^{de}	35.04±0.39^{cd}	35.62±0.49^{bc}
15	18.00±0.31^{d}	18.38±0.30^{d}	34.26±0.32^{a}	35.84±0.22^{a}	35.66±0.26^{cd}	35.18±0.35^{cd}	36.32±0.39^{abc}
20	22.32±0.55^{c}	22.10±0.29^{c}	34.14±0.40^{a}	35.00±0.32^{a}	36.44±0.27^{bc}	35.92±0.20^{bc}	36.80±0.76^{abc}
25	25.52±0.34^{b}	25.28±0.83^{b}	34.52±0.61^{a}	35.26±0.37^{a}	36.72±0.24^{ab}	36.64±0.22^{ab}	37.46±0.14^{ab}
30	28.20±0.44^{a}	30.80±1.04^{a}	35.40±0.07^{a}	35.96±0.31^{a}	37.28±0.16^{ab}	37.44±0.07^{a}	37.64±0.05^{a}
35	-	-	34.86±0.23^{a}	35.62±0.25^{a}	37.46±0.15^{a}	37.78±0.09^{a}	37.60±0.15^{a}

Date analysis use one-way ANOVA. Different superscript latters represent significant differences among the values in the same row, $P < 0.05$.

Table 2. The RMR of *A. chevrieri* pups that after exposure to different ambient temperature for 1h during postnatal development

Ambient temperature(℃)	Resting metabolic rate ($mLO_2/g\cdot h$)						
	1d	7d	14d	21d	28d	35d	42d
5	0.58±0.12^{e}	1.02±0.07^{d}	2.17±0.30^{b}	6.04±0.48^{a}	8.58±0.27^{a}	8.28±0.33^{a}	8.76±0.19^{a}
10	1.20±0.28^{de}	1.35±0.07^{cd}	1.97.±0.10^{b}	5.22±0.69^{a}	7.02±0.41^{b}	6.97±0.46^{b}	7.48±0.30^{b}
15	1.38±0.26^{cd}	1.63±0.30^{c}	3.25±0.17^{a}	3.04±0.53^{b}	5.72±0.36^{c}	6.14±0.36^{bc}	6.53±0.34^{c}
20	2.00±0.21^{bc}	2.36±0.09^{b}	3.08±0.13^{a}	2.46±0.24^{b}	3.64±0.15^{d}	4.95±0.44^{cd}	5.75±0.36^{c}
25	2.71±0.25^{b}	2.71±0.09^{b}	2.29±0.08^{b}	3.68±0.46^{b}	3.06±0.23^{d}	3.89±0.29^{cde}	4.38±0.24^{cd}
30	3.78±0.16^{a}	4.29±0.44^{a}	1.76±0.15^{bc}	3.47±0.31^{b}	2.84±0.26^{d}	4.26±0.48^{de}	4.90±0.26^{de}
35	-	-	1.34±0.20^{c}	2.80±0.47^{b}	3.56±0.54^{d}	5.54±0.57^{e}	6.20±0.35^{e}

Date analysis use one-way ANOVA. Different superscript latters represent significant differences among the values in the same row, $P < 0.05$.

DISCUSSION

Body temperature

Body heat insulation capability is very poor in pups of *A. chevrieri* when they were born, and started to grow at the age of 7 days, body temperature increased when with ambient temperature increase before the age of 14 days, showing similar characteristics of poikilotherm (Herczeg et al. 2006; Loman 2002; Reading 2003). Temperature regulation is the process of energy consumption, the pups of altricial animal at the early stage of development can not be effectively carried out physiological thermoregulation, rely mainly on parental care or other means to maintain body temperature, in order to reduce the energy utilization in the regulation of body temperature, which will be increased relatively higher energy for growing (Hull 1973). At the age of 14 days, pups showed weak body temperature regulation, lower body temperature at 5 and 10°C, which showed significant differences compared with other temperatures, body temperature had no significant difference after 15°C. After the 21 day, body temperature of pups appeared stable during environmental temperature changes. To the 28 day and 35 day old, its constant capacity has greatly improved compared to the 1 day and 7 day old; at the age of 42 days, body temperature of pups were relatively stable, showing constant capacity.

Pups in *A. chevrieri* establishing the ability of the constant thermoregulation capacity showed significant stage before 14 day of age, similar to many altricial mammals, such as *Peromyscus leucopus* (Hill 1983) and *Meriones unguiculatus* (McManus 1971). Animals from birth to the establishment of a thermostat mechanism is a gradual process, often during a thermoregulatory ability to rapidly mature stage (Blumberg and Sokoloff 1998; Sun and Zeng 1987), 7-14-day-old pups were rapidly period developing the ability to regulate body temperature, during which time it is fast grow development of body mass in pups, pups maintain a high body temperature in early development, which can prevent hypothermia caused by the growth slows down.

RMR

At the age of 1 and 7 days , RMR of pups increased as ambient temperature increases, which exhibited similar characteristics of poikilotherm (Angilletta et al. 2002; Navas et al. 2008), although this period the heat capacity is lower of pups at low temperatures (5-20°C), but they can still maintain the body temperature above ambient temperature, which can be inferred that pups had the ability to improve heat production respond to low temperature. In the present study, pups started to increased thermogenesis after 14-day-old, pups of 14-day-old also began to exhibit a constant body temperature. At the age of 21 days old, RMR of the pups were significantly higher at 5,10°C than when other points, indicating pups has been able to make certain thermal regulation responded to lower temperature, which was different to the immature mammals. The results of this experiment confirmed that many of the previous study concluded that altricial mammal is not completely molded variable temperature (Vinter et al. 1982).

At the ambient temperatures were higher(30-35°C), RMR of pups showed relatively higher in 1-7-day period, and it showed a downward trend in 14 and 21 days, RMR increased after 28 days of age, it can determine the thermal neutral zone between 22.5-30°C after 35 days, which close to adult levels (Zhu et al. 2008). This could be adapted to the Hengduan mountain region: Hengduan mountain region distributed in subtropical plateau, showing smaller year temperature difference, larger diurnal temperature difference, it is necessary to go through high temperature stress, while also experiencing cold stress, heat regulation and development capabilities are critical to the survival of pups in *A. chevrieri*.

In conclusion, pups of *A. chevrieri* in the different growing period had different thermogenesis and energy allocation to maintain stable to body temperature, thermogenesis was weaker in the early phase of postnatal development, most of energy is used to its growth. After pups were weaned, the ability of constant temperature and thermoregulation developed quickly to adjust variations of environment during postnatal development.

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