Journal of Thermal Biology 37 (2012) 316–322

Contents lists available at ScienceDirect

Journal of Thermal Biology

journal homepage: www.elsevier.com/locate/jtherbio

Simultaneous telemetric monitoring of the circadian changes in core and BAT temperature in rats: Endogenous vasopressin may contribute to reduced BAT themogenesis and body temperature in the light phase of the circadian cycle

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ARTICLE INFO

Available online 22 June 2011

Keywords:
Core temperature
Circadian rhythm
Brown adipose tissue
Arginine vasopressin
V1a-receptor antagonist
Lipid metabolism

ABSTRACT

The purpose of the present study was to analyze simultaneously the temporal relationship between the changes of circadian rhythms of brown adipose tissue (BAT) thermogenesis and core temperature (T_c) by dual probe telemetric monitoring transmitters and to determine the role of endogenous arginine vasopressin (AVP) in the circadian rhythms of BAT temperature (T_{BAT}) and T_c in male rats. The key observations in this study are: (1) Increase in T_{BAT} commenced approximately 8 min before T_c increases at the start of transition from the light to dark phase. Whereas at the start of transition from the dark to light phase, decrease in T_{BAT} commenced approximately 3 min before T_c decreases. The data show that circadian changes of BAT thermogenesis do indeed play a significant role in the overall maintenance of the circadian rhythm of core temperature. (2) The plasma AVP level was significantly elevated when core temperature decreases during the light phase, suggesting that endogenous AVP is involved in thermoregulatory processes during the light phase. V1a receptor antagonist could elevate core and BAT temperature during the light period, suggesting that endogenous AVP, acting through V1a receptor, could be involved in tonic thermoregulatory processes.V1a receptor antagonist can increase the blood lipid metabolism, suggesting that the mechanism of endogenous AVP in tonic thermoregulatory processes during light period could involve the suppression of lipolysis in BAT and other peripheral tissues. In summary, this study demonstrated that endogenous vasopressin contributes to reduced BAT themogenesis and body temperature in the light phase of the circadian cycle.

1. Introduction

Circadian rhythms of core temperature (T_c) result from endogenous rhythms of heat production and heat loss (Castillo et al., 2005). In rats and other nocturnal species, T_c increases during the night when activity increases, exhibiting the highest temperatures during the dark period and the lowest temperatures during the light period (Gordon, 1993; Castillo et al., 2005; Refinetti, 2010). Thermogenesis is a dual-purpose process, which helps to maintain the body temperature and contributes to the elimination of excess energy. Brown adipose tissue (BAT) has developed as an essential thermoregulatory effector in cold defense in rodents and other small mammals (Golozoubova et al., 2006), including infant humans. Several recent observations using positron emission tomographic scanning to assess tissue glucose uptake have demonstrated a remarkable amount of brown adipose tissue in adult humans, and the locations of BAT depots in adult humans bear a striking similarity to those in rodents (Cypess et al. 2009; Enerbäck, 2010).

Circadian rhythms of T_c and activity in mammals are generated by the suprachiasmatic nucleus of the anterior hypothalamus (Saleh et al., 1997; Castillo et al., 2005). It has been reported that endogenous arginine vasopressin (AVP) within the suprachiasmatic nucleus plays an important role in the generation of overt circadian rhythms (Ingram et al., 1998; Li et al., 2009). Few studies have also assessed the mechanism by which AVP evokes the drop in normal body temperature (Shido et al., 1984; Paro et al., 2003). We have found that chlorpyrifos-induced hypothermia was blocked by administration of V_1 receptors antagonist, suggesting that the thermoregulatory response to chlorpyrifos is mediated by central and/or systemic AVP release (Yang and Gordon, 2002).

However, previous studies were performed using the single probe transmitters, core and BAT temperature (T_{BAT}) could not be simultaneously measured telemetrically within the same animal. These studies required separate surgical implantations using different groups of animals to record changes in T_c and T_{BAT}. In addition,

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doi:10.1016/j.jtherbio.2011.06.007

many researchers use microchip transponders that measure subcutaneous temperature (Gordon et al, 2008). But subcutaneous temperature is not a measure of the core temperature; personnel must be in close proximity to animal to position receiver to collect temperature data; data collection not automated, no 24 h monitoring (Gordon et al, 2008). The latest development in telemetric monitoring is dual probe transmitters that record both T_c and T_{BAT} simultaneously in the same rat (Data Sciences International, Model TL10M2-F40-TT). A major advantage of simultaneous measuring T_c and T_{BAT} in the same animal is the ability to relate temporal changes on these two temperature parameters.

Therefore, the present study was first to determine whether BAT thermogenesis contributes to increase in T_c during the dark phase by simultaneous measurement of T_c and T_{BAT} in the same animal. Subsequently, we examined also the role of endogenous AVP in circadian rhythms of T_c and T_{BAT} in the rat.

2. Materials and Methods

2.1. Animals and drugs

Experiments were performed on adult male Sprague-Dawley rats weighting 230–310 g (Institute of Laboratory Animal Sciences, Sichuan Academy of Medical Sciences, China). Rats were housed individually in acrylic cages lined with wood shavings and maintained at an ambient temperature of 22 °C, and exposed to a daily 12:12 light: dark photoperiod (lights on at 06:00 h). Animals were allowed free access to water and food. All animal studies were complied with the WHO Guidelines of Humane Use and Care of Animals and approved by Institutional Animal Use and Care Committee.

V1a vasopressin receptor antagonist [beta-Mercapto-beta, beta-cycloentame-thylenepropionyl(1),O-Me-Tyr(2), Arg(8)]-VP were purchased from Sigma Chemical Co. (St Louis, MO). The drugs were dissolved in pyrogen-free sterile saline into a stock solution of 30 μg/ml and stored at −30 °C until the day of an experiment.

2.2. Surgery

T_c, T_{BAT} and motor activity was simultaneously measured in undisturbed rats using dual probe transmitters body temperature probe (Data Sciences International, Model TL10M2-F40-TT). The rats were anesthetized with sodium pentobarbital (40 mg/kg, intraperitoneally). The transmitter body and dual probe transmitters body temperature probe were placed in the abdominal cavity. A small hole was made in the side of the abdominal wall and the tip of interscapular BAT temperature probe was passed through this incision. The tip of the temperature probe (148 mm wire lead with a 3 mm tip diameter) was threaded out subcutaneously to position under interscapular BAT (Shido et al., 1984). In animals used for simultaneous measurement of T_c, axillary temperature (T_{ax}) and motor activity, the transmitter body and dual probe transmitters body temperature probe were implanted intra-abdominally as described above. The tip of T_{ax} probe was placed subcutaneously in the region of the axilla. Following surgery, rats were administered a penicillin antibiotic (20,000 units; intramuscularly). The rats were allowed at least 7 days of recovery before testing.

2.3. Measurement of T_c, T_{BAT}, and T_{ax}

T_c, T_{BAT}, and T_{ax}, and motor activity were monitored in undisturbed rats using radiotelemetry (Data Sciences International, St. Paul, MN, USA). The output of the transmitter was monitored at 5 min intervals by a receiver board placed under each rat's cage. The rat's motor activity was measured from the change in position of the transmitter in relation to the antennae located in the receiver board. Data were monitored on line as well as stored on computer for later analysis.

2.4. Analysis of the temporal relationship of BAT temperature increases to core temperature increases

T_{BAT}, T_c, and motor activity were simultaneously measured with a dual probe telemetric monitoring transmitters (DSI, Model TL10M2-F40-TT) in the same animal. The telemetry parameters were monitored at 1 min. intervals from rats housed individually in the animal facility. We analyzed the temporal relationship between BAT temperature and core temperature by calculating the mean value of each 1 min time point at the start of transition from the light to dark phase or at the start of transition from the dark to light phase, and determined the time difference between onset of the increase in BAT temperature and onset of the increase in the core temperature.

2.5. Experimental protocol

In all experimental protocols, the environmental chamber was set at 22 °C. The rats were placed in clean cages with wood shavings at 16:00 h. The rats were allowed to adapt to the chamber overnight. The following day the rats were dosed intraperitoneally with the saline or 30 μg/kg V1a receptor antagonist (0.1 ml/100 g body weight) at either 10:00 or 22:00 h. T_c, T_{BAT},T_{ax}, and motor activity of the rat were monitored by radio telemetry for at least 36 h prior to dosing. The rats were allowed to recover for at least 10 days and then they were retested using a crossover design where the rats that had received V1a receptor antagonist were given saline and the rats that had received saline were V1a receptor antagonist.

2.6. Plasma AVP measurements

Plasma levels of AVP were assessed in adult male Sprague-Dawley rats. The rats were housed individually and left undisturbed in the laboratory overnight with food and water provided ad libitum. Plasma AVP concentration was measured by an enzyme-linked immunosorbant assay based on a colorimetric reaction read at 405 nm using a 96-well microplate reader (Biotek Instrauments, Inc, Winooski, VT, USA). Blood was drawn by right ventricle puncture into a chilled EDTA-treated tube during the light phase betwen12:00–12:30 h, and during dark phase between 00:00–00:30 h. Plasma was separated in a refrigerated centrifuge (5 °C, 4000*g* for 15 min) and stored at −30 °C until analyzed for AVP. The AVP-RIA kit was obtained from Cayman Chemical Company (Ann Arbor, Michigan, USA).

2.7. Blood biochemical measurement

Adult male Sprague-Dawley rats were housed individually and left undisturbed in the laboratory overnight with food and water provided ad libitum. At 10:00 h the rats were dosed intraperitoneally with either saline or 30 μg/kg V1a receptor antagonist. Serum samples were collected from the right ventricle in rats at 5 h after V1a receptor antagonist. Because administration with 30 μg/kg V1a receptor antagonist at10:00 h led to a significant increase in T_c and T_{BAT} that persisted to the nocturnal phase approached (namely, persisted for over 8 h), the biochemical measurements were made on plasma taken 5 h after the administration of the drug. Serum was separated in a refrigerated centrifuge (5 °C, 4000*g* for 15 min) and was frozen at −30 °C. Serum triglyceride (TG), free fatty acid (FFA), glycerol, and ketone bodies were measured using the triglyceride E-test kit, FFA C-test kits, glycerol kit, and ketonic bodies E-test kit,

respectively. All assay kits were purchased from Cayman Chemical Company (Ann Arbor, Michigan, USA).

2.8. Statistical analysis

The 5 min telemetry data were averaged into 30 min bins for graphic presentation. The 1 min telemetry data were assessed the temporal relationship of BAT temperature increases to core temperature increases. Data were plotted as mean ± S.E. Values of T_c, T_{BAT}, and T_{ax} are the changes from basal values. Data were analyzed statistically by two-way ANOVA followed by multi-range (or others) test to assess differences between groups. Core temperature and motor activity data were analyzed over the 2 h period starting from the time of injection of drugs. This time period represents the time of maximum change in core temperature and motor activity for drugs. Blood levels of vasopressin and blood lipids were analyzed using a two-tailed Student's *t*-test. Values of $P < 0.05$ were considered to be significantly different.

3. Results

3.1. Temporal relationship of circadian rhythm of T_c, T_{BAT}, and T_{ax}

Fig. 1 shows that time course for the circadian rhythm of T_c, T_{BAT}, and motor activity was simultaneously measured in undisturbed rats using dual probe transmitters temperature probe during a 12 h light:12 h dark photoperiod. T_{BAT} was 0.67 °C lower than T_c during the light phase. The mean T_{BAT} and T_c were 36.66 ± 0.09 °C and 37.33 ± 0.06 °C, respectively. But T_{BAT} was similar to that T_c during the dark phase. The mean T_{BAT} and T_c of the rats were 37.98 ± 0.05 and 38.13 ± 0.08 °C, respectively. The amplitude of the circadian T_{BAT} rhythm was greater than simultaneous measurement of T_c (1.33 ± 0.06 and 0.92 ± 0.04 °C, respectively, Figs. 1 and 2A). It was noted that the rate of increase in T_{BAT} was higher than corresponding increases in T_c at the start of transition from the light to dark phase (Figs. 1 and 2B), and increase in T_{BAT} commenced approximately 8 min before T_c increases (Fig. 3). Whereas at the start of transition from the dark to light phase, decrease in T_{BAT} commenced approximately 3 min before T_c decreases (Fig. 3).

The amplitude of the circadian T_{ax} rhythm was similar to that of T_c (1.09 ± 0.03 and 0.91 ± 0.02 °C, respectively). During either the light phase or dark phase, T_{ax} was lower than simultaneous measurement of T_c (0.82 and 0.53 °C, respectively, Fig. 4). The rate of increase in T_{ax} was also similar to that T_c at the start of transition from the light to dark phase (Fig. 2B); however, the rate of increase in T_{ax} was lower than corresponding increase in T_{BAT} (Fig. 2B).

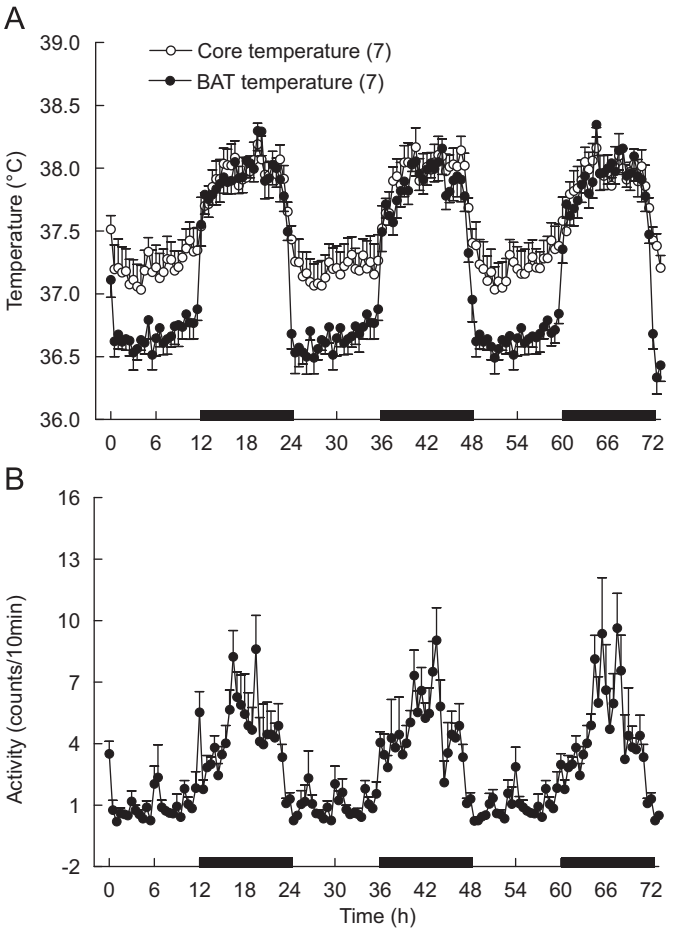

Fig. 1. Simultaneous telemetric monitoring of the time course of T_c, T_{BAT} (A) and motor activity (B) in male Sprague-Dawley rats over a 72 h period while housed at an ambient temperature (Ta) of 22 °C in the animal facility. Each point represents the mean of all temperatures recorded during every 30 min. In all figures, number in parenthesis indicate sample size, data were plotted as mean ± S.E. and dark horizontal bars represent the dark phase of the photoperiod.

3.2. Effect of V1a receptor antagonist on circadian rhythms of T_c and T_{BAT}

Administration of saline and V1a receptor antagonist led to a transient elevation in T_c and T_{BAT} that was attributed to the

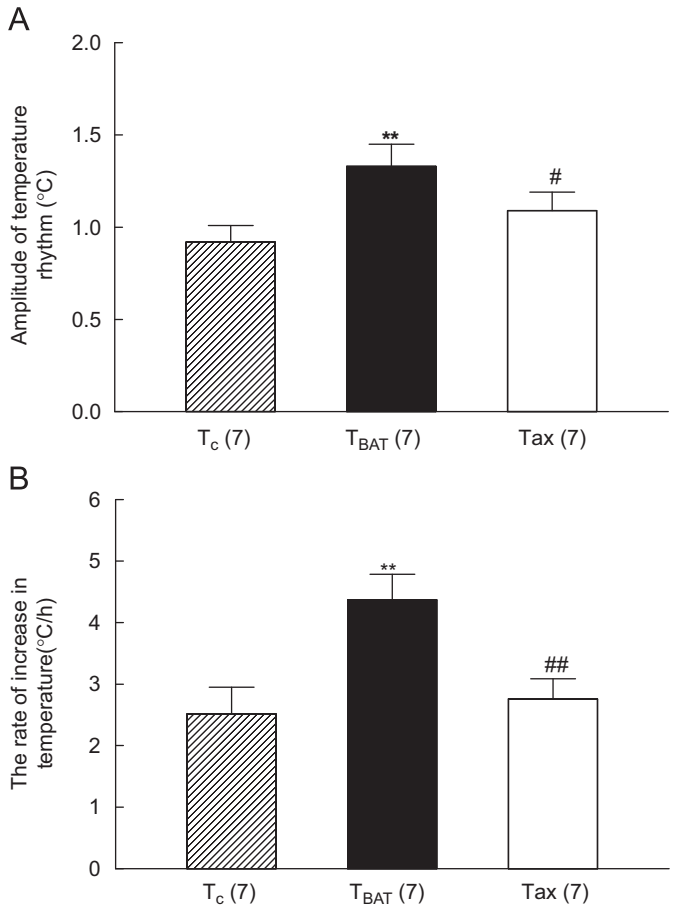

Fig. 2. Amplitudes of T_c, T_{BAT} and T_{ax} increases during the dark period (A). The rate of increases in T_c, T_{BAT} and T_{ax} (°C/h) at the start of transition from the light to dark phase (B).
**P < 0.01 when compared to core temperature; #P < 0.05, ##P < 0.01 when compared to BAT temperature.

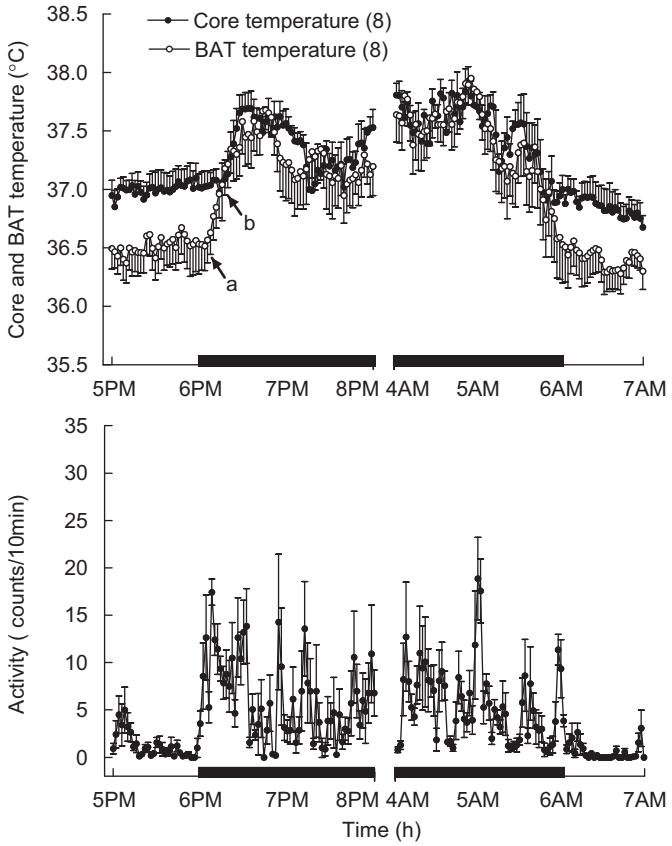

Fig. 3. Simultaneous telemetric monitoring of the time course of T_c, T_{BAT} and motor activity recorded at 1 min intervals. At the start of transition from the light to dark phase, the T_{BAT} (a) commenced 8 min before T_c (b) increases, and increases in T_{BAT} were larger than corresponding increases in T_c. Whereas decrease in T_{BAT} commenced approximately 3 min before T_c decreases at the start of transition from the dark to light phase. Data were plotted as mean ± S.E in 8 male rats.

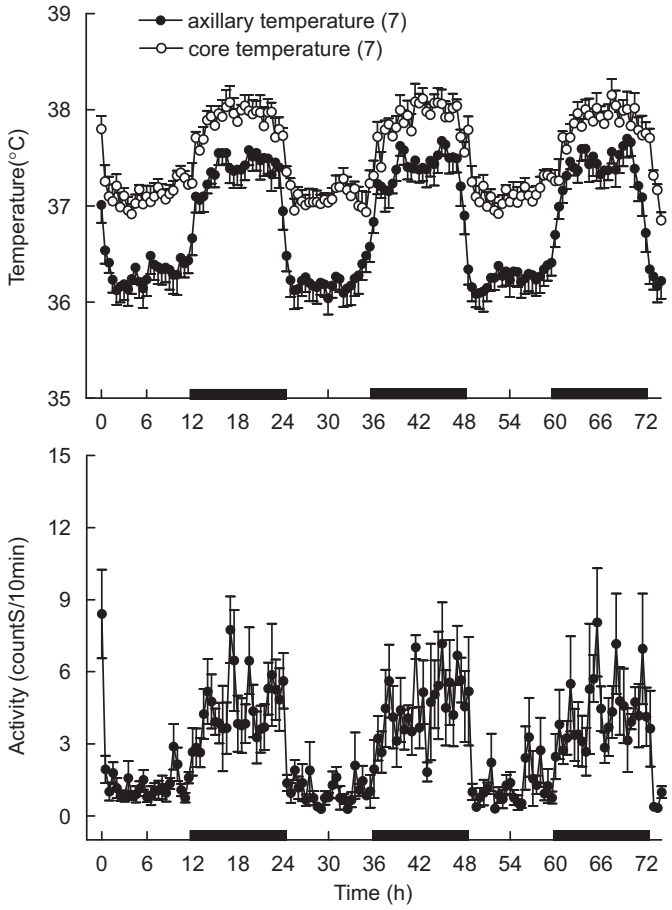

Fig. 4. Simultaneous telemetric monitoring of the time course of T_c, T_{ax} and motor activity in male Sprague-Dawley rats over a 72 h period while housed at a Ta of 22 °C in the animal facility.

handling and injection procedure (Fig. 5). Approximately 1.5 h after dosing, T_c of the saline group returned to near baseline levels.

During light phase (light on at 06:00–18:00 h), at10:00 h dosing with 30 μg/kg V1a receptor antagonist led to a significant increase in T_c that persist to the nocturnal phase approached (namely, persisted for over 8 h; Fig. 5 and Fig. 6). Their T_c was 0.31 °C higher than that of the rats given saline ($p < 0.01$). At the same time, V1a receptor antagonist had similar effects on T_{BAT} (Figs. 5 and 6). The mean T_{BAT} was 0.43 °C greater than control group ($p < 0.01$). During dark phase (light off at 18:00–06:00 h), at 22:00 h dosing with the same dose of V1a receptor antagonist had no significant effects on T_c and T_{BAT} (Fig. 7).

3.3. Light- dark difference of the AVP concentration in the plasma

AVP concentration in the plasma was 96.24 ± 31.16 pg/ml at the midday (12:00–12:30 h) during the light phase; at midnight (00:00–00:30 h) during the dark phase, the plasma AVP level was 55.6 ± 14.04 pg/ml (Fig. 8). The plasma AVP level was significantly elevated at the midday when compared to the serum AVP levels at the midnight ($p < 0.05$).

3.4. Effect of V1a receptor antagonist on serum lipid levels

To investigate effect of V1a receptor antagonist on the lipid metabolism, serum glycerol, triacylglycerol (TG) and free fatty acid (FFA) levels were examined 5 h after rats dosed with V1a receptor antagonist or saline. The levels of serum TG and FFA were significantly reduced by V1a receptor antagonist, whereas serum glycerol concentration was significantly higher than that of saline rats, indicating that V1a receptor antagonist enhanced significantly those catabolisms (Table 1). The final products of the lipid metabolism in blood, namely, ketone bodies including acetoacetic acid and 3-hydroxybutyric acid in serum, were also measured 5 h after V1a receptor antagonist. The serum levels of acetoacetic acid, 3-hydroxybutyric acid, and total ketone bodies were slightly, but not significantly, higher than that of saline rats (Table 1).

4. Discussion

This study measures simultaneously of the time course for the circadian rhythm of core and BAT temperature and to analyze their temporal relationship. The data show an overall circadian pattern of BAT temperature that agrees with the core temperature of the rat, and is in agreement with previous studies using the thermocouple sensors (Closa et al, 1993). It must be pointed out, however,that our data were obtained from simultaneous measurement of core and BAT temperature in the same animal by dual probe telemetric monitoring transmitters.

The normal physiological function of BAT thermogenesis is to heat the rest of the body. Episodic increases in core and brain temperature during the active period of the circadian cycle have previously been reported for rats and other species (Baker et al, 2005; Ootsuka et al, 2009). Recently, Ootsuka et al. (2009) found that increases in T_{BAT} occurred in an irregular episodic manner

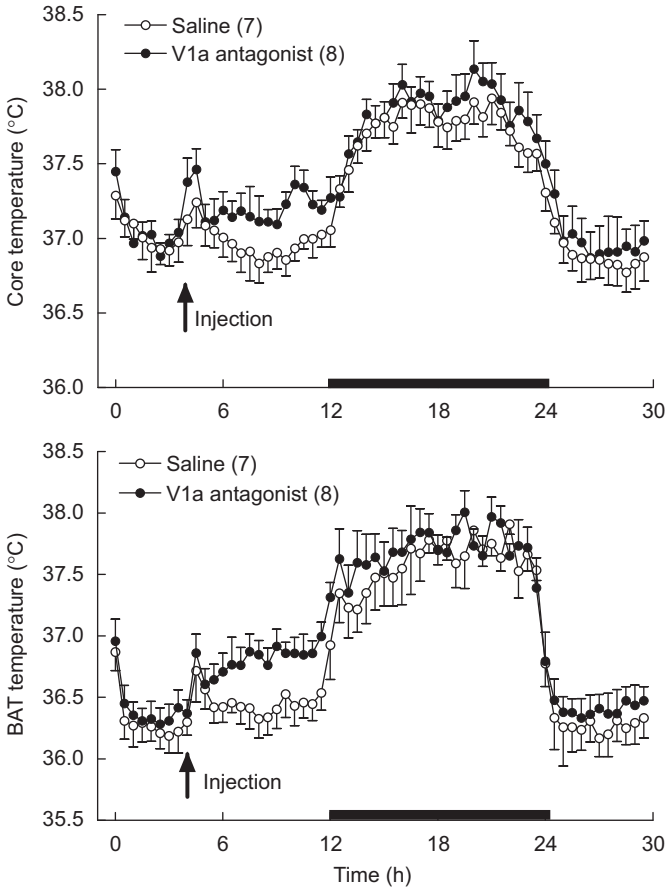

Fig. 5. Time-course of T_c and T_{BAT} at 10:00 h intraperitoneal administration of saline or 30 μg/kg V1a receptor antagonist. ANOVA analysis for saline comparing effects of V1a receptor antagonist treatment for 8 h: core temperature (treatment, $p < 0.01$; treatment-time, $p < 0.01$); BAT temperature (treatment, $p < 0.005$; treatment time, $p < 0.005$).

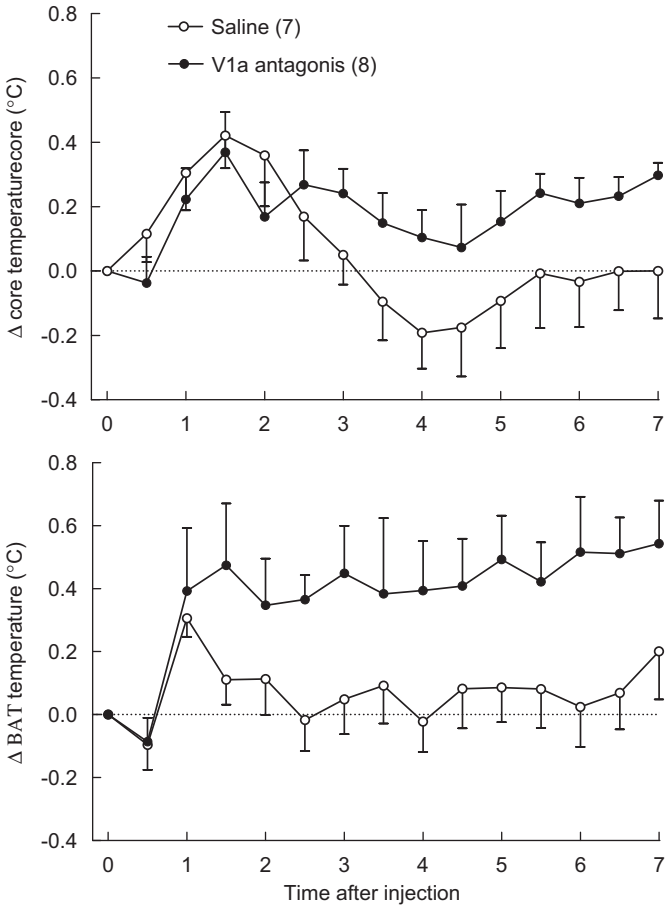

Fig. 6. Time-course of the change in T_c and T_{BAT} following intraperitoneal administration of saline or 30 μg/kg V1a receptor antagonist. Temperature changes calculated from data measured at the time of injection (see Fig. 5).

every 94 ± 43 min, the T_{BAT} episodes commenced 2–3 min before body temperatures episodes during the waking (dark) phase of the circadian cycle in rats, suggesting that episodic BAT thermogenesis contributes to episodic increases in body temperatures.

In the present study, we measured the temporal relationship between BAT temperature and core temperature at the start of transition from the light to dark phase or at the start of transition from the dark to light phase. Analysis of onset times of core temperature and corresponding increases in BAT temperature showed that increase in BAT temperature commenced approximately 8 min before body temperature at the start of transition from the light to dark phase, and the rate of increase in BAT temperature was larger than corresponding increases in core temperature. Whereas at the start of transition from the dark to light phase, decrease in BAT temperature commenced approximately 3 min before core temperature decreases.

It will be noted that BAT is located under the skin and measuring its temperature is a bit tricky because it is in a cooler region of the body and not in the true core of the animal. We therefore consider that comparison of BAT temperature with axillary temperature would determine whether BAT thermogenesis warms body. In this study, we showed that temperature probes are both being positioned under the skin but the BAT temperature probe is obviously showing much warmer temperatures compared to axillary temperature, suggesting that BAT themogenesis heats body in the dark phase of the circadian cycle. The data also suggest that radiotelemetry technique does indeed detect the heat produced by BAT.

In addition, microchip transponders that can encode animal identification number and temperature can be implanted under the skin in unanesthetized rodents (Gordon et al, 2008). Some studies have reported that microchip transponders measured a subcutaneous tissue temperature, which was in close agreement with rectal temperature using the digital rectal thermometer (Cilia et al, 1998; Quimby et al, 2009). The authors concluded that subcutaneous body temperature is representative of core body temperature (Cilia et al, 1998). Interestingly, our results demonstrate that overall circadian pattern of axillary temperature, measured via radiotelemetry, was lower than core temperature.

These results strongly suggest that BAT thermogenesis contributes to increase in core temperature during the dark phase, indicating that circadian changes of BAT thermogenesis does indeed play a significant role in the overall maintenance of the circadian rhythm of core temperature.

The increases in BAT temperature could reflect increases in BAT metabolic thermogenesis. BAT can increase its metabolic rate many times, although the tissue corresponding to only a few percent of the body weight, increases in BAT metabolism can substantially increase whole body metabolic rate (Cannon and Nedergaard, 2004). Thus increases in BAT metabolism could contribute substantially to the well-documented ultradian rhythmicity in whole body metabolic rate in rats during the dark active phase of the circadian cycle (Stupfel et al., 1995; Ootsuka et al., 2009).

It has been shown that AVP plays an important role in thermoregulation, because it is one of the main endogenous antipyretic molecules in the central nervous system (Chen et al., 1997).

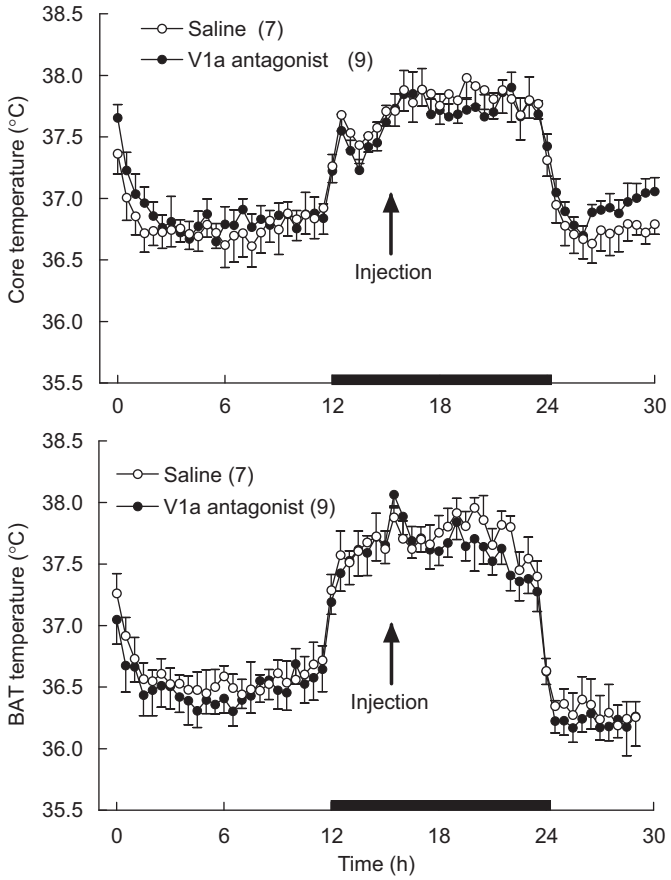

Fig. 7. Time-course of T_c and T_{BAT} following 22:00 h intraperitoneal administration of saline or 30 μg/kg V1a receptor antagonist. ANOVA analysis for saline comparing effects of V1a receptor antagonist treatment for 8 h: core temperature (not significant); BAT temperature (not significant).

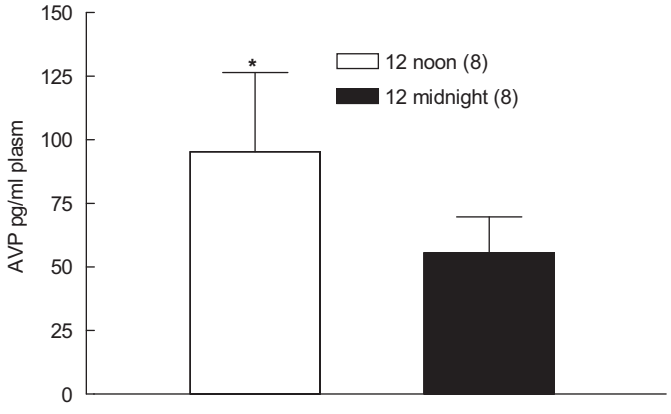

Fig. 8. Plasma AVP levels of male Sprague-Dawley rats. Data are mean ± S.E. of 8 rats.
*p < 0.05, significant difference compared to 24:00 h.

Hyperthermia can be elicited in rats by cooling of the preoptic area, which results in a vigorous metabolic response. In such animals, infusion of AVP into the lateral septum completely suppresses the metabolic response and hyperthermia (Pittman et al., 1998). Several studies have assessed the mechanism by which AVP evokes the drop in T_c. Paro et al. (2003) reported that ionotropic receptors of L-glutamate in the central nervous system participate in peripheral AVP-induced hypothermia by affecting heat loss through the tail. On the other hand, evidence suggests that hypothermia induced by AVP is brought about by a

Table 1
Mean ± S.E. of serum lipid metabolite concentrations taken from rats 5 h after treatment with V1a receptor antagonist or saline.

	V1a receptor antagonist group	Saline group
Triacylglycero l (μmol/L)	516.3 ± 40.8(7)**	735.9 ± 67.5(7)
Free fatty acid (μmol/L)	85.74 ± 6. 3(8)*	128.2 ± 10.6(8)
Glycerol (μmol/L)	473.9 ± 34.3(7)**	339.4 ± 29.1(8)
Total ketone bodies (μmol/L)	170.7 ± 15.7(6)	138.6 ± 27.2(6)
Acetoacetic acid (μmol/L)	34.1 ± 5.2(6)	27.7 ± 4.7(6)
3-hydroxybutyric acid (μmol/L)	136.6 ± 16.3(6)	110.9 ± 23.25(6)

* $P < 0.05$.
** $P < 0.01$ when compared to saline group.

coordinated response of reduced thermogenesis in BAT and increased heat loss through the tail (Shido et al., 1984; Paro et al., 2003).The physiologic effects of AVP are mediated by V_1 receptors (Richmond, 2003; Li et al., 2009). In addition, recent studies found that AVP signaling plays an important role in the generation of circadian rhythms (Ingram et al., 1998; Li et al., 2009).

However, mechanisms of the role of endogenous AVP in circadian rhythms of core temperature are still poorly understood. Therefore, we examined the role of endogenous AVP in circadian changes of core temperature in the male rats and effect of V1a receptor antagonist on the lipid metabolism. Administration of the V1a receptor antagonist led to a significant elevation in core and BAT temperature that persisted to the nocturnal phase approached at 10:00 h during light phase. In other words, this hyperthermic response in rats lasted for over 8 h. Ferris et al. (1988) reported that peptide V1 receptor antagonists blockade of AVP V1 receptor in hamsters lasts for over 12 h. Nonpeptide V_1 vasopressin receptor antagonist inhibits AVP-induced vascular smooth muscle cell contraction and blood pressure elevation for at least 8 h (Thibonnier et al., 1999). Whereas, administration of the V1a receptor antagonist had no effect on core and BAT temperature during dark phase. On the other hand, Plasma AVP level was significantly elevated at the midday when compared to the plasma AVP levels at the midnight. Previous studies have demonstrated that plasma AVP concentrations were higher during light period than during dark period (Greeley et al., 1982).

The results of this study provided two important pieces of evidence, which suggest that endogenous AVP may be playing a crucial role in the circadian changes of core temperature in the male rats. Firstly, the plasma AVP level was significantly elevated when core temperature decreases during the light phase, suggesting that endogenous AVP is involved in thermoregulatory processes during the light phase. Secondly, V1a receptor antagonist could elevate core and BAT temperature during the light period, suggesting that endogenous AVP, acting through V1a receptor, could be involved in tonic thermoregulatory processes. Interestingly, BAT temperature was slightly higher than core temperature after administration of V1a receptor antagonist, suggesting that endogenous AVP is likely to play a tonic role by reducing BAT thermogenesis.

There is increasing evidence to suggest that AVP plays an important role in the regulation of the lipid metabolism and other energy substrates (Rofe and Williamson, 1983; Kurihara et al., 1996; Hiroyama et al., 2007). In vitro, AVP inhibited forskolin-induced lipolysis in human adipocytes (Xue et al., 1998). It has been reported that the lipid metabolism is enhanced in *V1aR−/−* mice under the fasting condition (Hiroyama et al., 2007).

To examine the relationship between endogenous AVP involved in tonic thermoregulatory processes during light period and the suppression of lipolysis, we analyzed the effect of V1a

receptor antagonist injected peripherally on triacylglycerol (TG), free fatty acid (FFA), glycerol and ketone bodies levels in serum under the feeding condition. The intraperitoneal doses of V1a receptor antagonist can increase the glycerol level in serum, suggesting that lipolysis is promoted under a condition of abolished AVP via the V1a receptor. The increased circulating glycerol could be due to increased production, which results from the increased activity of tissue lipase (Hiroyama et al., 2007). Lipase mediates a breakdown of TG into glycerol and FFA. The FFA then serves as a precursor of ketoacids. Furthermore, V1a receptor antagonist can decrease the levels of TG and FFA in serum, suggesting that the catabolisms of TG and FFA were enhanced, in good correspondence with the increased glycerol levels in serum. This would suggest that the mechanism of endogenous AVP in tonic thermoregulatory processes during light period could involve the suppression of lipolysis in the BAT and other peripheral tissues.

However, under feeding conditions, serum levels of the ketone bodies after administration of V_1 a receptor antagonist were slightly, but not statistically, higher than that of rats given saline; corroborating another study showing that the serum levels of the ketone bodies were not significantly different in *V1aR+/+and V1aR−/−* mice under the feeding condition (Hiroyama et al., 2007). Previous studies have reported that AVP infusions decreased circulating ketone bodies in starved rats, suggesting that AVP has an antilipolytic action on adipocytes (Rofe and Williamson, 1983). Recently, Hiroyama et al. (2007) found that the serum levels of total ketone bodies were significantly increased in *V1aR−/−* mice under the fasting condition. It is possible that under fasting and starvation conditions, plasma levels of ketone bodies are higher than that under feeding conditions (Leung, 1995; Fukao et al., 2004). Ketone bodies are synthesized in the liver from acetyl-CoA, and under fasting/starvation conditions, lipid stores are mobilized, resulting in an increase of acetyl CoA production (Fukao et al., 2004).

In summary, This study is the first to analyze simultaneously the temporal relationship for the circadian rhythm in core and BAT temperature. The data show that circadian changes of BAT thermogenesis does indeed play significant role in the overall maintenance of the circadian rhythm of core temperature. Subsequently, we examined the role of endogenous AVP in the circadian rhythms of core and BAT temperature in the rat. The present work resulted in three principal findings: (1) plasma AVP concentrations were higher during light period than during dark period, (2) endogenous AVP, acting through V1a receptor, could be involved in tonic thermoregulatory processes in the male rat, and (3) V1a receptor antagonist can increase the blood lipid metabolism. These results strongly suggest that endogenous AVP is involved in the circadian changes of core temperature in the male rats.

Acknowledgments

We would like thank Dr. Christopher J. Gordon at U.S. Environmental Protection Agency for his review of the manuscript. This work was supported by grants from National Natural Science Foundation of China (No.30870901).

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