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Behavioral hypothermia of a domesticated lizard under treatment of the hypometabolic agent 3-iodothyronamine

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Abstract: Ectothermic animals rely on behavioral thermoregulation due to low capacity of heat production and storage. Previously, lizards were shown to achieve ‘fever’ during microbial infection by increasing their preferred body temperature (PBT) behaviorally, thereby attaining a relatively high survival rate. The purpose of this study was to investigate whether domesticated lizards pursued ‘behavioral hypothermia’ induced by a hypometabolic agent 3-iodothyronamine (T1AM). We found that treatment with 8.0 mg/kg T1AM caused a lizard species, the leopard gecko (*Eublepharis macularius*), to decrease its ventilation and oxygen consumption rates 0.64- and 0.76-fold, respectively, compared to those of the control ($P < 0.05$). The lizards, habituated at an ambient temperature of $30 \pm 0.5^\circ\text{C}$, also showed a significant decrease in the PBT range over a freely accessible thermal gradient between 5°C and 45°C . The upper limit of the PBT in the treated lizards lowered from 31.9°C to 30.6°C , and the lower limit from 29.5°C to 26.3°C ($P < 0.001$). These findings demonstrate that the treated lizards pursued behavioral hypothermia in conjunction with hypoventilation and hypometabolism. Because prior studies reported a similar hypometabolic response in T1AM-injected laboratory mice, the domesticated lizards, as a part of the vertebrate phylogeny, may be a useful laboratory model for biological and pharmacological researches such as drug potency test.

Key words: 3-iodothyronamine, behavioral hypothermia, lizards, metabolism, thermoregulation

Introduction

The regulation of body temperature (T_b) is crucial for animals to maintain homeostasis to ensure optimal function and a high probability of survival [5, 7, 17]. Although a large difference exists in physiological thermoregulatory capacity (e.g., heat generation and storage) between ectothermic and endothermic vertebrates, the principal mechanism of thermoregulation appears to be shared in the vertebrate phylogeny [2, 17, 19]. Fever and hypothermia are special physiological mechanisms for

survival in critical conditions such as those resulting from microbial infection or external stressors [4, 16]. The two mechanisms may thus provide a good opportunity to examine the hypothesis that the basis of thermoregulation is conserved in the vertebrates. A representative example is seen in fever, a form of hyperthermia produced by the endogenous pyrogens of the host in response to bacterial endotoxins. An important finding is that the desert iguana (*Dipsosaurus dorsalis*) induced fever after bacterial infection by raising their preferred body temperature (PBT) behaviorally, leading to an

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increase in survival rate [18]. By analogy with behavioral fever in lizards, we may ask whether ectotherms voluntarily induce 'behavioral hypothermia' in accordance with a well-defined hypometabolic state (see below).

Previous reports have shown that hypometabolism and hypothermia can be induced in non-hibernator rodents by administration of brain extracts from hibernating animals [15] or of certain chemicals, such as 3-iodothyronamine (T1AM) and H₂S [1, 14]. For instance, mice treated with a single dose of T1AM reduced their metabolic rate until T_b decreased to ~30°C over the first 90 ~ 120 min of exposure [11]. Normothermia was then gradually restored 4 to 6 h after injection. Another comparable example is seen in the administration of H₂S to mice. Interestingly, the same substance (in the form of Na₂S) also produced hypometabolic and hypoventilatory effects in a fish, the blue cod (*Parapercis colias*) [6]. In the fish study, however, because the water (thus body) temperature was maintained at a constant value, it is not known whether the individuals would voluntarily reduce T_b to produce a hypometabolic response.

In the current study, we injected T1AM into the leopard gecko (*Eublepharis macularius*) to investigate whether the chemical affects the metabolic and thermoregulatory activity of this organism. T1AM is known to be naturally produced by the enzymatic deiodination and decarboxylation of thyroid hormone T4 [13]. T1AM has been found to trigger a hypometabolic response of target cells via a specific transmembrane receptor such as G-protein-coupled receptor (e.g., trace amine-associated receptor 1, TAAR1) [14]. As deiodinase, decarboxylase and TAARs are found in most vertebrates [8–10], it is presumed that the interaction of T1AM with the receptor is conserved in the vertebrates. We therefore tested the hypothesis that the T1AM-treated lizards induce hypothermia behaviorally over a freely exploitable thermal gradient. The results of this study may fill the gap in the current knowledge of the fundamental thermoregulatory mechanism of the ectotherms. The results will also give an opportunity to examine whether the domesticated reptiles can be used as a laboratory model for biological and pharmacological researches. These issues have been overlooked to date.

Material and Methods

Animals

This study was carried out in accordance with the Guide for the Animal Care and Use of the Committee on the Ethics of Animal Experiments of Yonsei University Wonju Campus. The protocol was approved by the Committee on the Ethics of Animal Experiments of Yonsei University (Permit Number: YWC-130115-2). Leopard geckos (*E. macularius*; 18.9 ± 2.1 g, n=26, 1 year old) were purchased from a local supplier. Before the experiments, all the subjects were habituated for a week under a light:dark cycle of 12:12 h with lights on 07:00, a relative humidity of ~40% and a cage temperature of 30 ± 0.5°C maintained with a heating pad under one side of the cage. The animals were fed daily with a diet of gut-loaded mealworms supplied at night. All the experiments were conducted after 19:00 as these geckos are nocturnal.

Ventilation rate

To determine an appropriate dose of T1AM, the ventilation rate (VR, counts per min) was first tested at four different doses injected intraperitoneally (i.p.) in 2 – 3 subjects per group: 0 mg/kg (vehicle control), 4 mg/kg, 8 mg/kg and 16 mg/kg. Each of the doses was dissolved in 60% dimethyl sulfoxide (DMSO) and 40% saline (pH 7.4) [14]. The VR was measured by counting the number of thoracic movements every 10 min for the first hour and subsequently every 30 min until the end of the experiment. The dose of 8 mg/kg T1AM was chosen as an appropriate one because the subjects did not further decrease the VR at 16 versus 8 mg/kg (see below in the Results), and because prior observations showed that rodent subjects often died at relatively high doses during experiments, possibly due to a prolonged bradycardia [14]. Once the protocol was decided, the VR was determined with six to eight lizards by injecting the vehicle or T1AM.

Oxygen consumption rate

The general procedure for measurements of oxygen consumption rate (VO₂) was described in our previous reports [11, 12]. The six lizards were fasted for 24 h before the experiment. An individual subject was placed in a 340-ml metabolic chamber set at 30 ± 0.5°C. The subject was stabilized for 1 h in the chamber and was injected i.p. with either DMSO or T1AM (8 mg kg⁻¹).

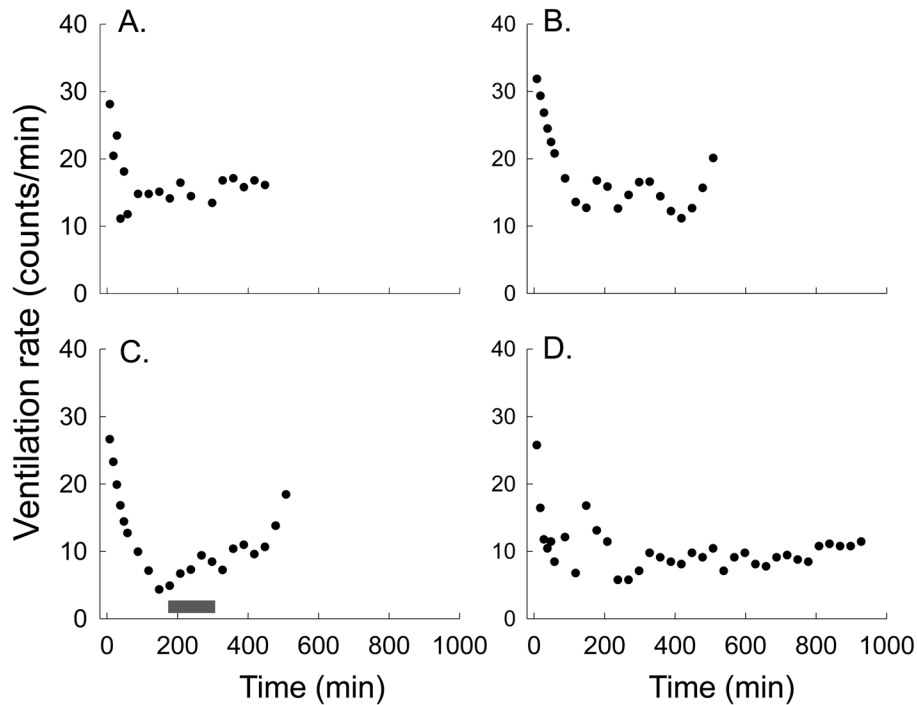


Fig. 1. Representative records of ventilation rates of leopard geckos treated with only DMSO (vehicle control) (A) or T1AM at dosages (mg/kg) 4.0 (B), 8.0 (C) and 16.0 (D). Data of the time period from 180 to 300 min were used for the statistical analysis (indicated in C with a gray bar on the x-axis).

The VO_2 data were collected every 10 min with an Oxy-max System (Columbus Instruments, Columbus, OH, USA) that included an automated, open-circuit equal-flow system connected to the metabolic chamber. The incurrent air supplied to the chamber was set at 550 ml/min. Excurrent air subsamples were collected at 500 ml/min, diverted through desiccant columns and sent to the O_2 sensor. The gas analyzer signals were transmitted to a personal computer. The VO_2 was calculated according to a Hill's equation, expressed as a mass-specific value and corrected to standard temperature (0°C) and pressure (760 Torr).

Preferred body temperature

The PBT of the lizards was measured over a thermal gradient between 5°C and 45°C set up on a copper plate ($W \times L \times T = 70 \text{ mm} \times 905 \text{ mm} \times 5 \text{ mm}$). This plate was surrounded by 170-mm high acrylic plates to isolate the subjects from the external environment. To generate the thermal gradient, one end of the copper plate was warmed by a controlled heat block and the other end cooled by a circulator containing an anti-freeze mixture. After the setting was completed, the T_b of each lizard was monitored

with a 49 AWG (0.025 mm diameter) duplex copper-constantan thermocouple (California Fine Wire, Grover City, CA, USA) connected to a digital thermometer (Cole-Palmer Instrument, Vernon Hills, IL, USA). The sensing tip of the thermocouple was inserted 7 mm deep into the latissimus dorsi muscle. The subject was then injected i.p. with the vehicle or T1AM, and was placed randomly on either the warm or the cool end of the copper plate.

Statistical analysis

The data are presented as mean \pm SD. The significance of differences in VR between groups was tested via independent samples *t*-tests. Differences in VO_2 and PBT between treatments were assessed via paired-samples *t*-tests. The procedure was performed with SPSS/PC+, and significance was accepted at the level of $P < 0.05$.

Results

The VR of the control animals stabilized at a level of 13 to 17 CPM within 30 min of DMSO injection (Fig. 1). The VR decreased slightly further at a dose of 4 mg/kg T1AM, but decreased considerably to 4 ~ 10

Table 1. Summary data for ventilation rate, oxygen consumption rate and upper and lower limits of preferred body temperature in leopard gecko

| Variables | Control ² | T1AM ² |
|---|--------------------------|--------------------------|
| Ventilation rate (counts/min) | | |
| Average VR* | 11.6 ± 1.9 | 7.4 ± 1.6 |
| ¹ Minimum VR* | 8.9 ± 2.1 (n = 6) | 3.0 ± 2.8 (n = 8) |
| Oxygen consumption rate [†] (ml/g/h) | 0.075 ± 0.011 (n = 6) | 0.056 ± 0.014 (n = 6) |
| Preferred body temperature (°C) | | |
| Upper limit (UL) [‡] | 31.9 ± 0.5 | 30.6 ± 1.7 |
| Lower limit (LL) [‡] | 29.5 ± 1.3 | 26.3 ± 1.1 |
| | | (n = 6) |

Data: mean ± SD. ¹Minimum VR is the mean of the single lowest VRs of individual subjects over 180 to 300 min post-injection. ²Significance of differences between DMSO and T1AM treatments: *, $P < 0.001$ (independent t -test); [†], $P = 0.03$ (paired t -test); [‡], $P < 0.001$ (paired t -test).

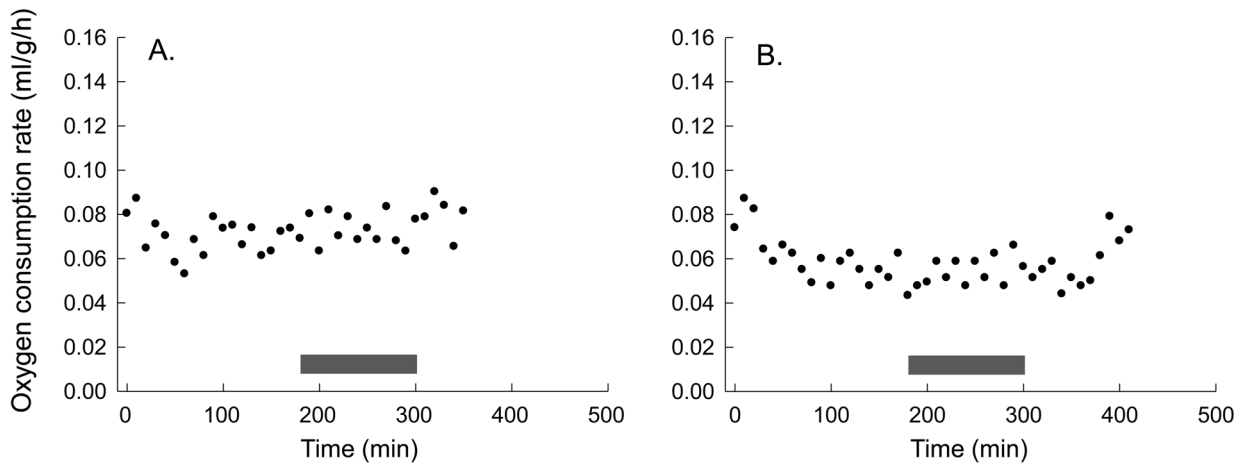


Fig. 2. Typical records of oxygen consumption rates (VO_2) for a gecko injected with vehicle (A) or T1AM (B). Note that the subject in either treatment showed relatively stable levels of VO_2 for 180 to 300 min (indicated with gray bars) that were averaged and used as a representative value for that measurement.

counts/min at 8 mg/kg. After 400 min post-injection, the VR at these doses returned to a value at or above the control level. The VR remained at 5 ~ 9 counts/min for up to 930 min at a dose of 16 mg/kg T1AM (Fig. 1D). From these results, we chose a dose of 8 mg/kg as a suitable dosage of T1AM and a 2-h time frame from 180 min to 300 min for the subsequent analyses. Under the influence of T1AM, the average and minimum VR decreased 0.64- and 0.33-fold, respectively, compared with the control VRs (independent t -test, $P < 0.001$) (Table 1).

The VO_2 was determined using six subjects treated with and without T1AM (Fig. 2). The average VO_2 was 0.072 when the animals were treated with only the vehicle, but it decreased 0.76-fold for the same animals

injected with T1AM (paired t -test, $P = 0.03$) (Table 1).

Figure 3 illustrates the experimental apparatus with a gecko on a copper plate that displayed a thermal gradient between 5°C and 45°C (A to C). Figures 3D and 3E presents typical T_b readings over ~400 min post-injection for two animals, each with and without T1AM treatment. The T_b profile was nearly identical in both conditions for up to 180 min post-injection. The T_b values subsequently diverged, with the subjects treated with T1AM showing lower T_b ranges. With the T1AM treatment, the average upper limit (UL) of the PBT decreased from 31.9° to 30.6°C, and the lower limit (LL) decreased from 29.5° to 26.3°C (paired t -test for both UL and LL, $P < 0.001$) (Table 1).

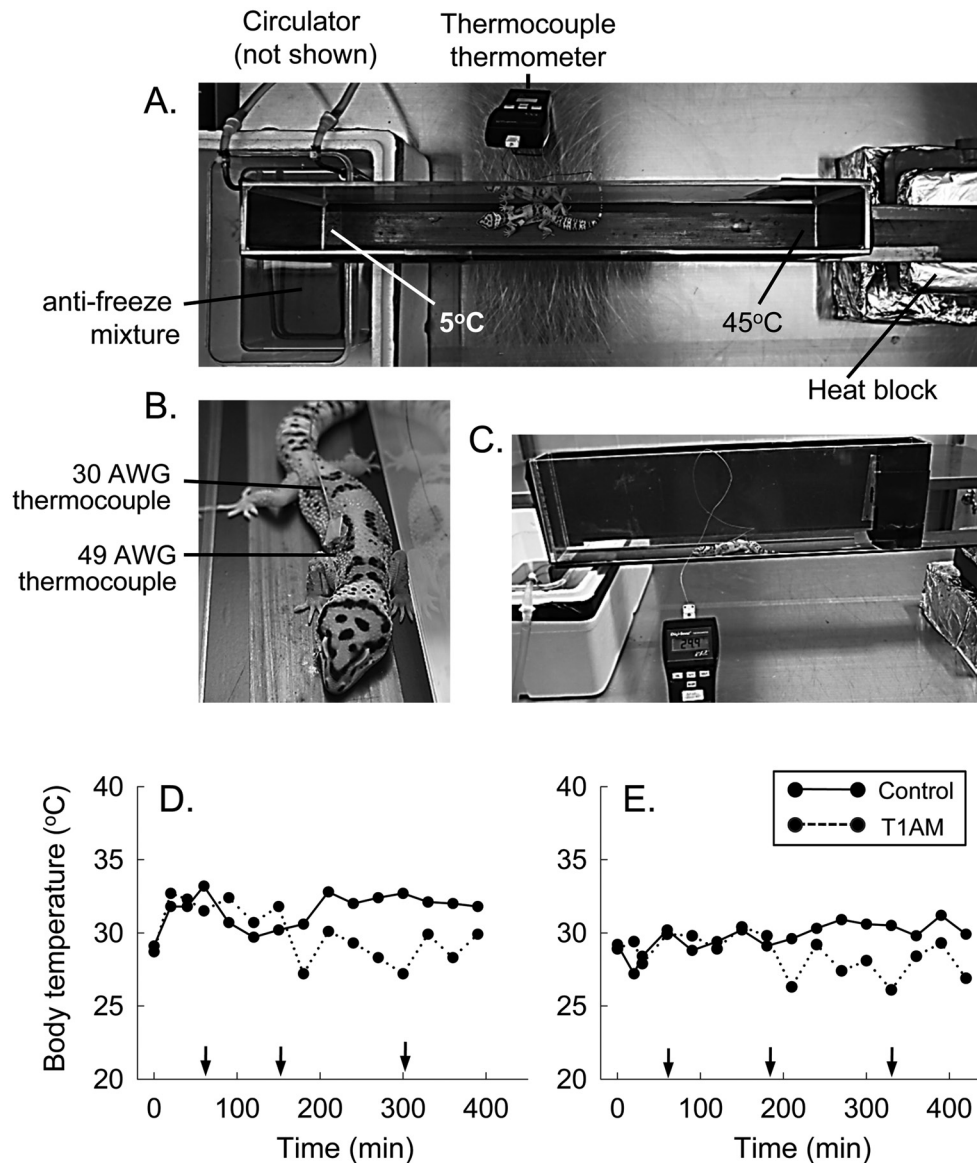


Fig. 3. An experimental setting for measurements of the PBT of the lizards over a thermal gradient between 5°C and 45°C. (A) Top view showing the whole apparatus. (B – C) A gecko moving along the copper plate. The sensing tip of the 49 AWG (0.025 mm diameter) thermocouple (barely visible) connected to the 30 AWG thermocouple was inserted into a dorsal muscle to measure its T_b . (D and E) Typical records of T_b monitored from two lizards. Each lizard was tested on the copper plate with injection of DMSO (control) or T1AM (see the details in Materials and Methods). A subject shown in D was placed on the warmest end of the copper plate (45°C); a subject shown in E was placed on the coolest end of the plate (5°C). Black arrows on both the x-axes show the time points at which the subject moved to other thermal regions.

Discussion

Ectotherms respond to thermoregulatory demands primarily by behavioral means. These demands are adaptive since the optimal T_b causes increases in reproductive, digestive and performance capacity [7, 20]. The

behavioral mode of response is observed even in the special case of fever in the lizard *D. dorsalis*. In this situation, the PBT range shifted to a higher level in the presence of a bacterial infection, resulting in a higher survival rate [18]. Thus, in light of the fundamental principle of thermoregulation, we investigated whether ec-

totherms show the ability to attain hypothermia behaviorally.

We found that T1AM was effective for inducing hypoventilation and hypometabolism in the geckos. The average VR and VO₂ were reduced 0.64- to 0.76-fold as a result of T1AM treatment (Table 1). These results suggest that the reptiles might have the same cellular mechanism to that of rodents for interaction of T1AM with its membrane receptors which then trigger hypometabolism. It is worth noting that the quantity of T1AM required for the induction of hypometabolism in lizards was 1/6.25 of the quantity required in mice (8 vs. 50 mg/kg) [14]. This difference may partly reflect the low VO₂ of the lizards compared to mice (0.072 vs. 1.93 ml/g/h) at the standard or normal T_b [11].

Our final goal was to investigate whether the T1AM-induced hypometabolism was accompanied by behavioral hypothermia. The results of the experiment supported our hypothesis. The experiment furnished the first evidence that both the upper and lower limits of the PBT in lizards treated with T1AM were significantly lower than those of the same animals treated with only the vehicle DMSO. Because the subjects were free to move along the thermal gradient, it appeared that hypothermia was pursued behaviorally under the T1AM-induced hypometabolic state. This differential change in the PBT occurred at 150 to 180 min post-injection, the time at which T1AM was fully effective for hypoventilation and hypometabolism (Figs. 1 and 2). Conversely, our results may imply that the lizards exploit a relatively low thermal environment and remain in a low T_b range, as do mammalian hibernators, when reduction of metabolic rate is crucial for survival (e.g., during times of food shortage) [2, 3].

In conclusion, the T1AM-treated lizards voluntarily pursued behavioral hypothermia. This result is consistent with hypoventilation and hypometabolism. Although the adaptive significance of hypothermia was not directly explored in this study, a reduction of energy expenditure, with decreased VR and VO₂, would be beneficial to ectotherms in certain conditions like food shortage. Our result provides valuable evidence that the principal thermoregulatory mechanism might be conserved in the same phylogenetic lineage including the domesticated reptiles as well as laboratory mice.

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