Increased thermogenic capacity of brown adipose tissue under low temperature and its contribution to arousal from hibernation in Syrian hamsters

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Am J Physiol Regul Integr Comp Physiol 302: R118–R125, 2012. First published October 12, 2011; doi:10.1152/ajpregu.00053.2011.—Brown adipose tissue (BAT) is considered to play a significant physiological role during arousal from hibernation. Brown adipocytes are distributed in the interscapular and adrenal-gland regions and are thought to play a significant physiological role during arousal when body temperature rises from the extremely low body temperature that occurs during hibernation. The dominant pathway of BAT thermogenesis occurs through the β₃-adrenergic receptor. In this study, we investigated the role of the β₃-adrenergic system in BAT thermogenesis during arousal from hibernation in both in vitro and in vivo. Syrian hamsters in the hibernation group contained BAT that was significantly greater in overall mass, total protein, and thermogenic uncoupling protein-1 than BAT from the warm-acclimated group. Although the ability of the β₃-agonist CL316,243 to induce BAT thermogenesis at 36°C was no different between the hibernation and warm-acclimated groups, its maximum ratio over the basal value at 12°C in the hibernation group was significantly larger than that in the warm-acclimated group. Forskolin stimulation at 12°C produced equivalent BAT responses in these two groups. In vivo thermogenesis was assessed with the arousal time determined by the time course of BAT temperature or heart rate. Stimulation of BAT by CL316,243 significantly shortened the time of arousal from hibernation compared with that induced by vehicle alone, and it also induced arousal in deep hibernating animals. The β₃-agonist SR59230A inhibited arousal from hibernation either in part or completely. These results suggest that BAT in hibernating animals has potent thermogenic activity with a highly effective β₃-receptor mechanism at lower temperatures.

Thermogenesis in BAT is physiologically stimulated by norepinephrine released from sympathetic nerve terminals. Transduction of the thermogenic signal occurs mainly through β₃-adrenergic receptors on the membrane of brown adipocytes and is coupled by adenylyl cyclase activation, resulting in increased cytosolic cAMP levels (44, 45). Increased cAMP then induces hydrolysis of stored triacylglycerol by activating cAMP-dependent PKA. Through this process, free fatty acids are liberated as fuel for mitochondrial oxidation and simultaneously as activators of uncoupling protein-1 (UCP1), a key thermogenic protein in BAT (2).

Many aspects of BAT thermogenesis during arousal from hibernation have been investigated, such as activation of UCP1 (15), preferential blood flow to BAT (29), increased arousal rate by norepinephrine, and decreased arousal or inhibition of arousal by inhibitors of norepinephrine synthesis or broad β-blockers (7, 10, 11, 13, 22). However, despite these studies, little evidence of the physiological cellular mechanisms behind these processes has been obtained. The aim of this study was to clarify whether BAT is indispensable in thermogenesis during arousal from hibernation in mammals and whether the thermogenic capacity of BAT is maintained at low temperatures comparable to the body temperature of the hibernator. In addition, possible modification of the intracellular signal transduction pathway of brown adipocytes to induce thermogenesis during arousal from hibernation was investigated.

MATERIALS AND METHODS

Animals and Housing

The ethics committee of Asahikawa Medical University (ethical approval no. 10112) approved all of the animal experiments, which conformed to the ethics guidelines of the Japanese Physiological Society. Syrian hamsters (Mesocricetus auratus) were housed individually (12:12-h light-dark cycle; room temperature, 25 ± 2°C) with access to hamster chow and water ad libitum. After attaining a body weight of 100 g, the animals were transferred to a cold (5 ± 1°C) room with constant darkness and were again given access to food and water ad libitum. After 2 mo, the animals began to hibernate. Infrared motion sensors were used to monitor activity, and a computer program calculated the timing and duration of each hibernation bout. Regularly hibernating animals (>70 h/bout) were chosen for all experiments. For in vitro experiments, the following groups were used for the study: 1) warm-acclimated (W) animals, which were not transferred to the cold and constantly dark room; 2) cold-acclimated (C) animals, which had acclimated to the cold and constantly dark room for 2 wk but did not enter hibernation; and 3) hibernating (H) animals, which had entered deep hibernation under cold and dark conditions.
Reagents and Buffers

CL316,243 (CL; a specific β3-adrenergic receptor agonist), SR59230A (SR; a selective β3-adrenergic receptor antagonist), and forskolin were obtained from Sigma (St. Louis, MO). CL was dissolved in sterile PBS and forskolin was dissolved in DMSO. SR was dissolved in DMSO and then diluted in PBS to a final concentration of 1%. Buffers were prepared as described previously (38) and contained the following: Krebs-Ringer phosphate buffer (in mM: 148 Na+, 6.9 K+, 1.5 Ca2+, 1.4 Mg2+, 119 Cl−, 1.4 SO42−, 5.6 H2PO4−, 16.7 HPO42−, 10 glucose, 10 fructose, 4% crude BSA, pH 7.4); Krebs-Ringer bicarbonate buffer (in mM: 145 Na+, 6.0 K+, 2.5 Ca2+, 1.2 Mg2+, 128 Cl−, 1.2 SO42−, 25.3 HCO3−, 1.2 H2PO4−, 10 pyruvate, 10 glucose, 10 fructose, 4% fatty acid-free BSA, pH 7.4). Krebs-Ringer bicarbonate buffer was equilibrated with 5% CO2 in air.

Measurement of Oxygen Consumption

For in vitro experiments, animals were euthanized by exsanguination via cardiac puncture under Nembutal anesthesia (50 mg/kg ip), and interscapular and subscapular BATs were excised. Interscapular BAT was cleaned of any adhering tissue, weighed, and minced with scissors in Krebs-Ringer phosphate buffer at 37°C. Tissue blocks of the specimen (~1 mm3) were floated in Krebs-Ringer phosphate buffer and incubated for 1 h at 37°C with gentle shaking. After incubation, the buffer was changed, and oxygen consumption was measured. Samples before measurement were stored at 4°C. Oxygen consumption was measured using a Clark-type oxygen electrode (Rank Brothers, Cambridge, UK) at 36°C and 12°C in a water-jacketed chamber where ~5 to 10 mg of preincubated tissue was incubated at each temperature and stirred in 2 ml Krebs-Ringer bicarbonate buffer and 5% CO2 in air. After determination of the basal oxygen consumption, the stimulant (10 μl) was added to the incubation medium.

Protein Content and Western Blot Analysis

Subscapular BAT was cleaned of any adhering tissue, weighed, and homogenized in Tris-EDTA buffer (10 mM Tris, 1 mM EDTA, pH 7.4). After centrifugation at 800 g for 10 min at 4°C, the supernatant was collected for determining UCP1 and cytochrome oxidase subunit 4 (COX4). Protein content was determined by the bicinchoninic acid method with BSA as the standard. Antibodies against human UCP1 and human COX4 were obtained from Sigma and Cell Signaling Technology (Danvers, MA), respectively. Briefly, 10 μg of total protein was separated by SDS-PAGE and transferred to polyvinylidene fluoride membranes (Immobilon; Millipore, Bedford, MA). The membranes were incubated overnight in blocking buffer [50 mM Tris-HCl (pH 7.5), 150 mM NaCl] containing 0.1% Tween 20 and 5% skimmed milk, and then in the same buffer containing each antibody for 1 h at room temperature. The bound antibody was detected with horseradish peroxidase-conjugated goat anti-rabbit immunoglobulin (Amersham, Little Chalfont, UK) and an enhanced chemiluminescence system (Amersham). The relative intensity of UCPI and COX4 staining was determined using an National Institutes of Health Image J version 1.42.

Chronic Catheterization and Permanent Positioning of Silver Rings

When animals were cenothermic in the interbout arousal phase, chronic catheterization of the femoral vein was performed, and silver rings to be used for ECG recordings were permanently placed in the back skin under Nembutal (50 mg/kg ip) and regional Xylocaine anesthesia. Briefly, the tip of the silicon tube on the polyethylene catheter (PE-10), which was filled with sterile saline-heparin solution, was positioned in the femoral vein and the other side of the PE-10 catheter was exteriorized subcutaneously between the scapulae. The animals typically reentered hibernation within 7 days after surgery, after which the following experiments were performed.

In Vivo Experiments

To investigate the overall contribution of β3-adrenergic receptor to BAT function in the arousal mechanism of hibernation, the specific agonist (CL) or the selective antagonist (SR) was infused through an indwelling femoral vein catheter in the arousal and deep hibernation phases. Heart rate and/or BAT and rectal temperature were recorded to confirm the effects of the infusion.

Effect of CL and SR on Forced Arousal

Twenty hamsters with chronic catheterization were used in the experiment. Animals whose activity had not been detected for 40–60 h were forced into arousal by tactile stimulation, and temperature and heart rate were recorded. Heart rate was calculated by the R-R interval of the ECG, with the ECG leads connected to the permanently positioned silver rings. The back skin between the scapulae was cut under regional Xylocaine anesthesia and a fine-wire (0.1 mm) Cu-Ct thermocouple was inserted under the interscapular BAT and into the rectum. This procedure took < 5 min. After the heart rate was confirmed to be < 20 beats/min, cold PBS or CL (0.01 mg/ml) was infused via the femoral vein catheter at a rate of 5 μl/min for 200 min. In another set of experiments, when the BAT temperature (TBAT) reached 10°C, about 60–120 min after the tactile stimulation, 500 μl of cold vehicle (1% DMSO) or SR (2 mg/ml) was infused subcutaneously. If the animals failed to arouse 4 h after stimulation, intravenous injection of CL (0.2 μg/animal) was performed to rescue them from highly probable death.

Effect of CL in Deep Torpor

Seven hamsters with chronic catheterization were used in the experiment. When no activity was detected for 40–60 h, the ECG leads and syringe were gently connected to the exteriorized femoral vein catheter and to the permanently positioned silver rings, respectively, and the heart rate was recorded. After the heart rate was confirmed to be < 10 beats/min, cold PBS was infused via the catheter at a rate of 5 μl/min for 10 min. After 2 (n = 4) and 3 h (n = 3), cold CL solution (0.01 mg/ml) was infused in the same manner, and heart rate elevation was monitored.

Statistical analyses. Statistical analyses were performed using InStat software (GraphPad Software, San Diego, CA). Differences in heart rate, heart rate, and arousal time between the administration groups, in vivo, were analyzed using Student's t-test. Differences in body weight, tissue weight, protein content, and oxygen consumption of BAT between the experimental groups, in vitro, were determined using one-way ANOVA, followed by analysis using Tukey's post hoc test. P < 0.05 was considered statistically significant. The results were expressed as means ± SE.

RESULTS

Body Weight, Tissue Weight, and Protein Content of Interscapular BAT

Animals in deep hibernation had lost body weight compared with the warm-acclimated and cold-acclimated animals. Interscapular BAT weight per total tissue pad and per unit body weight was significantly increased in the hibernating animals compared with the warm-acclimated animals. The amount of UCP1 and the whole protein content were larger in both the cold-acclimated and hibernating animals than in the warm-acclimated animals (Table 1).

In Vitro Experiments

CL induces a thermogenic response of BAT at normothermic temperatures. A representative time course of oxygen consumption in response to stimulation by CL, measured at dif-
Table 1. **Body weight, tissue weight, and protein content of BAT**

<table>
<thead>
<tr>
<th></th>
<th>W, n = 12</th>
<th>C, n = 5</th>
<th>H, n = 9</th>
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</thead>
<tbody>
<tr>
<td>Body weight, g</td>
<td>109.8 ± 14.4</td>
<td>112.2 ± 18.1*</td>
<td>84.6 ± 7.1†</td>
</tr>
<tr>
<td>IBAT weight, mg</td>
<td>133.3 ± 23.6</td>
<td>181.6 ± 13.5*</td>
<td>232.9 ± 44.3†</td>
</tr>
<tr>
<td>per body weight, mg/100 g body wt</td>
<td>121.3 ± 16.3</td>
<td>164.7 ± 18.5*</td>
<td>274.5 ± 42.4†</td>
</tr>
<tr>
<td>BAT protein, μg/mg BAT</td>
<td>81.8 ± 12.7</td>
<td>171.0 ± 9.4*</td>
<td>143.4 ± 21.9†</td>
</tr>
<tr>
<td>per BAT, AU/mg BAT</td>
<td>1.2 ± 0.2</td>
<td>1.4 ± 0.2</td>
<td>1.6 ± 0.2*</td>
</tr>
<tr>
<td>COX4 protein, AU</td>
<td>100.8 ± 32.5</td>
<td>167.0 ± 41.6*</td>
<td>231.5 ± 49.7†</td>
</tr>
<tr>
<td>per BAT, AU/mg BAT</td>
<td>1.7 ± 0.9</td>
<td>1.7 ± 0.4</td>
<td>1.4 ± 0.4</td>
</tr>
</tbody>
</table>

Values are means ± SD. Brown adipose tissue (BAT) weight and protein were obtained from interscapular BAT (IBAT) and subscapular BAT. W, C, and H are warm-acclimated, cold-acclimated, and hibernating animals, respectively. Values of uncoupling protein-1 (UCP1) and cytochrome oxidase subunit 4 (COX4) proteins were estimated by the relative intensity of Western blot analysis staining. UCP1 and COX4 protein [arbitrary units (AU)] were calculated as a value for each unit amount of extracted total BAT protein. *P < 0.05 vs. W; †P < 0.05 vs. C.

Forskolin induces thermogenic response of BAT. Forskolin, a specific activator of adenylyl cyclase, was used to determine whether potent thermogenesis of BAT obtained from hibernating animals under hypothermic conditions was caused by modification in the downstream signaling pathway of cAMP. A summary of the responses to CL (1 μM) and forskolin (10 μM) in the oxygen consumption of BAT from each group of 4–5 animals is shown in Fig. 1C. No difference was observed after forskolin stimulation at 12°C between the warm-acclimated and hibernating animals. In addition, there were no significant differences at 36°C (data not shown).

In Vivo Experiments

**\( \beta_3 \)-Adrenergic stimulation.** The effect of CL administration on forced arousal from hibernation is summarized in Fig. 2A and Table 3, and the effect on deep hibernation is shown in Fig. 2B. As shown in Table 3, initial TBAT and heart rate did not differ between the vehicle- and CL-infused groups during arousal from hibernation. CL infusion during arousal significantly shortened the time to arousal (in this case, \( T_{BAT} = 27°C \) or heart rate = 200 beats/min) compared with vehicle infusion. Mean warming rates of TBAT calculated according to the stages of arousal were significantly higher only at the early stage (\( T_{BAT} \approx 10°C \)) in the CL-infused group.

To elucidate whether arousal by BAT thermogenesis occurs through the \( \beta_3 \)-adrenergic pathway, CL was administered to animals in deep hibernation. To avoid the possible interference of hibernation, we measured only heart rate through the chronically positioned silver rings; we did not measure temperature. As shown in the previous result of forced arousal from deep hibernation, both TBAT and heart rate changed almost simultaneously in the arousal phase of hibernation (Fig. 2) (28). To compare the results between vehicle and CL in deep hibernation, the change in heart rate when each injection time was aligned as time 0 is shown in the inserted graph in Fig. 2B. Regardless of when the vehicle was injected (−120 min or −180 min), no change in heart rate was observed, despite the possibility that the stimulus of vehicle injection itself disturbed the deep hibernation. On the contrary, CL significantly increased the heart rate within 50 min of the beginning of infusion compared with vehicle infusion (see graph in Fig. 2B), and resulted in arousal in all animals (heart rate > 200 beats/min in 4–5 h).

Fig. 1. Oxygen consumption of interscapular brown adipose tissue (BAT) measured at two different temperatures (36°C and 12°C). A: representative response of BAT from warm-acclimated animals to the \( \beta_3 \)-agonist CL316,243 (CL; 1 μM). The assays were performed at 36°C (●) and 12°C (○). B: mean maximum response to CL (1 μM) in BAT from animals that were warm acclimated (white bars), cold acclimated for 2 wk (black bars), and hibernating (gray bars). C: comparison of the effects of CL (1 μM) and forskolin (10 μM) on BAT from warm acclimated (white bars) and hibernating (gray bars). *P < 0.05 vs. warm acclimated; †P < 0.05 vs. cold acclimated. NS, not significant. Values are means ± SE.
Table 2. β3-agonist-induced oxygen consumption of interscapular BAT at different temperatures

<table>
<thead>
<tr>
<th></th>
<th>36°C</th>
<th>12°C</th>
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<tbody>
<tr>
<td></td>
<td>Basal</td>
<td>Max§</td>
</tr>
<tr>
<td>W</td>
<td>143.7 ± 15.6</td>
<td>220.1 ± 23.4</td>
</tr>
<tr>
<td>C</td>
<td>100.3 ± 12.6</td>
<td>133.6 ± 7.5*</td>
</tr>
<tr>
<td>H</td>
<td>88.6 ± 11.7*</td>
<td>122.9 ± 11.9*</td>
</tr>
<tr>
<td></td>
<td>18.2 ± 1.2</td>
<td>27.8 ± 1.5</td>
</tr>
<tr>
<td></td>
<td>18.0 ± 2.0</td>
<td>24.1 ± 0.9</td>
</tr>
<tr>
<td></td>
<td>21.4 ± 3.9</td>
<td>29.6 ± 4.4</td>
</tr>
</tbody>
</table>

Values are means ± SD. Basal oxygen consumption (Basal) was determined by the average of 5-min data before agonist administration. Maximum oxygen consumption (Max) induced by the β3-agonist (CL 316,243: 1 μM) was determined by the average of 5-min data 5 min or 15 min after stimulation at 12°C or 36°C, respectively, as shown in Figure 1A. §In every case, maximum values were significantly higher than basal values. *P < 0.05 vs. W.

DISCUSSION

The thermogenic capacity of BAT at low temperatures resembling the torpid state in hibernation was investigated in vitro. BAT from hibernating hamsters was large in size and had abundant UCP1 compared with that from warm-acclimated animals, as has been previously reported (9). BAT thermogenesis produced by the β3-adrenergic receptor agonist in hibernating animals was larger at 12°C than in nonhibernating animals; however, this was not observed at 36°C. This suggests that BAT of hibernating animals has high thermogenic capacity at low temperatures. BAT stimulation by the β3-agonist not only accelerated heat production during arousal from hibernation but also induced arousal when animals were in deep hibernation. On the other hand, BAT blockade by the β3-antagonist inhibited arousal from hibernation, strongly suggesting that arousal from hibernation requires β3-adrenergic stimulation, which may be related to the high thermogenic capacity of BAT.

In Vitro Experiments

As Syrian hamsters are facultative hibernators (23), they can enter into hibernation within 2–3 mo after exposure to the two specific environmental cues of cold temperature and a short photoperiod. In this study, 2 wk of continuous exposure to cold and short photoperiod increased the amount of BAT and UCP1 in these animals, thus supporting previous reports that either or both of these cues stimulate BAT growth and UCP1 production (5, 21, 27, 30, 33, 37, 41). Although UCP1 activity, as estimated by the amount of GDP bound to mitochondria, was found to be increasing even at 2 wk after exposure to 4°C, where it then reached a plateau in BAT (14); additional increases of BAT and UCP1 were observed in the animals that entered hibernation, suggesting that a greater thermogenic response is expected in BAT under such conditions.

Despite increased UCP1 in the hibernating animals, no significant increase in the maximum rate of oxygen consumption under normothermic conditions was observed. A similar
change in BAT respiration has been reported in nonhibernating as well as hibernating cold-acclimated animals (6, 24). This contradiction is now attributed to the desensitization of the β3-adrenergic receptor, and modifications involved in the receptor mechanisms including the postreceptor signaling pathway are speculated (34, 35, 38). Although such modification of the β3-pathway seems advantageous when animals maintain body temperature at a certain level, it is likely to be obstructive when a large amount of heat is required in a relatively short time, such as during arousal from hibernation. Thus, we hypothesized that BAT enables hibernating animals to generate heat efficiently at lower temperatures. As hypothesized, in the present study, BAT in hibernating animals responded to the β3-agonist more potently than that in warm- and cold-acclimated animals (Fig. 1B). Interestingly, BAT from cold-acclimated animals did not show a larger response than that from warm-acclimated animals. This suggests that, in addition to the amount of UCP1, some other factors may be involved in the potent thermogenesis observed in BAT during hibernation. Although it remains unclear how such high reactivity of BAT to the β3-agonist at low temperatures is achieved before the animal enters the hibernation season, it is possible to speculate from the results of the present experiment that utilized the specific activator of adenylyl cyclase, forskolin. We did not examine any dose-response relationships, but the data in Fig. 2, A and B clearly demonstrate that the β3-agonist acted as a trigger on arousal and mediated BAT thermogenesis at the early stage of arousal. It is somewhat interesting that this additional stimulation increased the warming rate of BAT, because full activation of the sympathetic nervous system does not seem to occur at the onset of arousal.

We found that β3-blockade only partially inhibited arousal. Feist et al. (7) also obtained a similar result using an inhibitor of norepinephrine synthesis. One probable explanation for the animals arousing normally from hibernation is the failure of the antagonist to distribute to critical sites. Another explanation is the condition of the animals; that is, even though in the present study all of the animals were injected with the antagonist when T_BAT reached 10°C, the time to reach this temperature varied substantially among the animals, meaning that the sympathetic nervous system activity may not have been the same in all animals. The fact that that animals whose T_BAT did not exceed 15°C (Fig. 3, E–G) and whose rectal temperature did not exceed 14°C (data not shown) failed to arouse supports this explanation. As Syrian hamsters start to shiver when their body...
who were able to shiver aroused successfully. Their arousal rate, however, is lower because of the blockade of NST.

The contribution of BAT to thermogenesis during arousal from hibernation has also been speculated. Although a UCP1-independent compensatory thermogenic mechanism mediated by sympathetic β3-receptors has been reported (26, 36), it is known that UCP1 can mediate adaptive NST potently and effectively in response to cold (8). Conclusive findings demonstrating the necessity of BAT during the arousal phase of hibernation was not acquired in this study, but the present results strongly imply that BAT function is indispensable for warming of the body in the early phase of arousal. The development of a reversible and highly selective method of blocking BAT/UCP1 function is required to conclusively determine whether BAT function is required. Genetic ablation of UCP1 in hamsters may be a candidate for such a method (26).

Although we found no direct evidence of the interaction between in vitro and in vivo experiments, it can be concluded that the high capacity of BAT thermogenesis under low temperatures contributes to successful arousal from hibernation. Future studies should clarify how such high thermogenic capacity at low temperatures can only be obtained through hibernation and not from 2 wk of cold acclimation, which is adequate for adaptation of UCP1 activity (14).

**Perspectives and Significance**

In hibernators, BAT functions under extremely low body temperature seem to be essential for arousal from hibernation. This importance of BAT functions may be conclusively revealed in the not-so-distant future by investigations using techniques such as gene knock out (26) and reversible and highly selective blocking of BAT/UCP1 function. Analysis of the regulation mechanism of BAT thermogenesis at the molecular level may also contribute to the investigation of this hibernation mechanism. In humans, on the other hand, despite the known existence of BAT in neonates, it has long been controversial whether adults have BAT. Recently, the existence of functional BAT in adults has been confirmed by using state-of-the-art methods (40, 42). Despite both the difficulty of BAT detection in obese persons (39) and the occurrence of compensatory NST in genetically BAT-ablated mice (26), it is suspected that BAT still remains important in nonhibernators, including humans, and it is estimated that > 50% of NST in response to cold stimulation is caused by BAT. In the hibernation process, BAT functions under extremely low body temperature to produce heat and maintain a high body temperature. The role of BAT in arousal from hibernation is essential, and future studies should elucidate how BAT functions contribute to successful arousal from hibernation.

![Graph](http://ajpregu.physiology.org/)

**Table 4. Arousal time determined by BAT temperature and HR by β3-antagonist**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Vehicle, n = 5</th>
<th>SR59230A, n = 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>BAT at time 0 (°C)</td>
<td>10.4 ± 0.5</td>
<td>10.2 ± 0.7</td>
</tr>
<tr>
<td>HR at time 0, beats/min</td>
<td>30.4 ± 4.1</td>
<td>33.2 ± 2.3</td>
</tr>
<tr>
<td>Time to reach BAT = 27°C, min</td>
<td>103.4 ± 20.8</td>
<td>255.6 ± 138.5*</td>
</tr>
<tr>
<td>Time to reach HR = 200 beats/min, min</td>
<td>97.2 ± 19.2</td>
<td>245.6 ± 139.6</td>
</tr>
<tr>
<td>Numbers of rescued animals</td>
<td>0 (30%)</td>
<td></td>
</tr>
<tr>
<td>Numbers of animals that died</td>
<td>0 (17%)</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SD. Animals in deep hibernation were aroused by the tactile stimulation and vehicle or the β3-antagonist (SR59230A: 1 mg/animal) were injected subcutaneously when BAT temperature reached 10°C (time 0). Arousal from hibernation was determined as in Table 3. Temperature and HR data were obtained from the successfully aroused animals (n = 5) including 2 successfully rescued animals perfused with CL (0.2 μg/animal iv) 4 h after antagonist injection (see MATERIALS AND METHODS). *P < 0.05 vs. vehicle.
nator, however, as the findings of the present study suggest, BAT is an indispensable organ for the cold-adaptation behavior of hibernation; it might be preferable for research on BAT function to be done in true hibernators because of this essentiality. Nonetheless, even if animal BAT is used, detailed investigation of BAT, an organ of wasted energy, can provide a clue for the treatment of human obesity.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

N.K. and M.H. performed experiments; N.K. and M.H. analyzed data; N.K. and M.H. prepared figures; N.K. and M.H. drafted manuscript; N.K. and M.H. approved final version of manuscript; M.H. conception and design of research; M.H. edited and revised manuscript.

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